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(54) Title: THERMOSTABLE PHYTASES IN FEED PREPARATION AND PLANT EXPRESSION

(57) Abstract

The use of thermostable phytases in the preparation of animal feed, and the expression in plants of such phytases. For preparation of animal feed, a thermostable phytase is added before or during the agglomeration step. Preferred processes are pelleting, extrusion and expansion. A transgenic plant expressing a thermostable phytase may be used diretly in animal feed preparation.

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Thermostable phytases in feed preparation and plant expression

Technical Field

This application relates to thermostable phytases, viz. their use in processes for the production of animal feed, and their expression in plants.

Background art

WO 91/14782 describes transgenic tobacco and rapeseed plants expressing a phytase derived from Aspergillus ficuum NRRL 3135. The transgenic tobacco seeds are fed to broilers.

US 5,824,779 describes in standard fashion how to produce transgenic alfalfa expressing the same A. ficuum phytase, and the preparation of a phytase-containing concentrate which can be used per se as an animal feed supplement.

EP 0 556 883 B1 describes a method for preparing feed pellets based on an extrusion technique. The addition of temperature sensitive agents, one example of which is phytase, takes place after extrusion of the feed pellets, and the sensitive agents are loaded onto the pellets under reduced pressure.

As acknowledged in EP 0 556 883 B1 the loss of activity of heat-sensitive substances during feed preparation processes is a well-known problem. The above EP-patent proposes to solve this problem by adding these substances under reduced pressure subsequent to the extrusion process. This solution, however, requires a liquid form of the sensitive substance, as well as the installation of additional expensive process equipment.

The present invention provides an improved process for preparing animal feed, as well as improved phytase-expressing transgenic plants.

Summary of the Invention

The present invention provides a process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the agglomeration.

Also provided is a transgenic plant or part thereof which comprises a DNA-construct encoding a thermostable phytase.

The transgenic plant or part thereof, e.g. seeds or leaves, may be used in the feed preparation process of the invention, to thereby provide - in a preferred embodiment - at the same time a nutrient (feed ingredient) and the feed additive phytase.

Brief description of the Figures

In the detailed description of the invention below, reference is made to the drawings, of which

Fig. 1 is a differential scanning calorimetry (DSC) chart of consensus phytase-1 and consensus phytase-10;

Fig. 2 a DSC of consensus phytase-10-thermo-Q50T and consensus phytase-10-thermo-Q50T-K91A;

Fig. 3 a DSC of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A;

Fig. 4 a DSC of the phytase from A. fumigatus ATCC 13073 and of its α-mutant; and

Fig. 5 shows the design of the consensus-phytase-1 amino acid sequence;

Fig. 6 an alignment and the basidiomycete consensus sequence of five Basidiomycete phytases;

Fig. 7 the design of the consensus-phytase-10 amino acid sequence;

Fig. 8 an alignment for the design of consensus-phytase-11 (all Basidiomycete phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycete sequence; still further the amino acid sequence of A. niger T213 was used);

Fig. 9 the DNA and amino acid sequence of consensus-phytase-1-thermo(8)-Q50T-K91A;

Fig. 10 the DNA and amino acid sequence of Consensus-phytase-10-thermo(3)-Q50T-K91A;

Fig. 11 the DNA and amino acid sequence of A. fumigatus ATCC 13073 α-mutant; and

Fig. 12 the DNA and amino acid sequence of Consensus-phytase-7 which comprises the following mutations as compared to Consensus-phytase-1: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

Detailed description of the invention

In the present context a "feed" or an "animal feed" means any natural or artificial diet, meal or the like intended or suitable for being eaten, taken in, digested, by an animal. Food for human beings is included in the above definition of feed.

"Animals" include all animals, be it polygastric animals (ruminants); or monogastric animals such as human beings,

poultry, swine and fish. Preferred animals are the mono-gastric animals, in particular pigs and broilers.

The concept of "feed ingredients" includes the raw materials from which a feed is to be, or is, produced; or the intended, or actual, component parts of a feed. Feed ingredients for non-human animals are usually, and preferably, selected from amongst the following non-exclusive list:

plant derived products

such as seeds, grains, leaves, roots, tubers, flowers, pods, husks - and they may take the form of flakes, cakes, grits, flour, and the like;

animal derived products

such as fish meal, milk powder, bone extract, meat extract, blood extract and the like;

additives

such as minerals, vitamins, aroma compounds, and feed enhancing enzymes.

Phytic acid or myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate (or for short myo-inositol hexakisphosphate) is the primary source of inositol and the primary storage form of phosphate in plant seeds and grains. In the seeds of legumes it accounts for about 70% of the phosphate content. Seeds, cereal grains and legumes are important feed ingredients.

Phytic acid, or its salts phytates - said terms being, unless otherwise indicated, in the present context used synonymously or at random - is an anti-nutritional factor. This is partly due to its binding of nutritionally essential ions such as calcium, trace minerals such as mangane, and also proteins (by electrostatic interaction). And partly due to the fact that the phosphorous thereof is not nutritionally available

either, since phytic acid and its salts, phytates, are often not metabolized.

This leads to a need of supplementing food and feed preparations with e.g. inorganic phosphate.

The non-metabolizable phytic acid phosphorous passes through the gastrointestinal tract of such animals and is excreted with the manure, resulting in an undesirable phosphate pollution of the environment resulting e.g. in eutrophication of the water environment and extensive growth of algae.

Phytic acid is degradable by phytases. In the present context a "phytase" is an polypeptide or enzyme which exhibits phytase activity, viz. which catalyzes the hydrolysis of phytate (myo-inositol hexakisphosphate) to (1) myo-inositol and/or (2) mono-, di-, tri-, tetra- and/or penta-phosphates thereof and (3) inorganic phosphate.

The production of phytases by plants as well as by microorganisms has been reported. Amongst the microorganisms, phytase producing bacteria as well as phytase producing fungi are known.

There are several descriptions of phytase producing filamentous fungi belonging to the fungal phylum of Ascomycota (ascomycetes). In particular, there are several references to phytase producing ascomycetes of the Aspergillus genus such as Aspergillus terreus (Yamada et al., 1986, Agric. Biol. Chem. 322:1275-1282). Also, the cloning and expression of the phytase gene from Aspergillus niger var. awamori has been described (Piddington et al., 1993, Gene 133:55-62). EP 0420358 describes the cloning and expression of a phytase of Aspergillus ficuum (niger). EP 0684313 describes the cloning and expression of phytases of the ascomycetes Aspergillus niger, Myceliophthora thermophila, Aspergillus terreus. Still further, some partial

sequences of phytases of Aspergillus nidulans, Talaromyces thermophilus, Aspergillus fumigatus and another strain of Aspergillus terreus are given.

The cloning and expression of a phytase of Thermomyces lanuginosus is described in WO 97/35017.

WO 98/28409 describes the cloning and expression of several basidiomycete phytases, e.g. from Peniophora lycii, Agrocybe pediades, Paxillus involutus and Trametes pubescens.

According to the Enzyme nomenclature database ExPASy (a repository of information relative to the nomenclature of enzymes primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) describing each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided), two different types of phytases are presently known: A so-called 3-phytase (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) and a so-called 6-phytase (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26). The 3-phytase hydrolyses first the ester bond at a 3-position, whereas the 6-phytase hydrolyzes first an ester bond at the 6-position of phytic acid. Both of these types of phytases are included in the above definition of phytase.

Many assays of phytase activity are known, and any of these can be used for the purpose of the present invention. Preferred phytase assays are included in the examples.

The concept of "agglomeration" is defined as a process in which various components are mixed under the influence of heat. The resulting product is preferably an "agglomerate" or conglomerate in which the components adhere to each other while forming a product of a satisfactory physical stability. The formation of dust from such agglomerate is an indication of its

physical stability - the less dust being formed, the better. A suitable assay for dust formation from agglomerates is ASAE standard S 269-1. A satisfactory agglomerate has below 20%, preferably below 15%, more preferably below 10%, even more preferably below 6% dust.

"Under the influence of heat" means that the temperature is at least 65°C, as measured on the product at the outlet of the agglomeration unit. More preferred temperatures are at least 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at least 130°C.

A preferred agglomeration process is operated at an increased pressure. The pressure is typically due to a compacting of the ingredients, optionally in combination with a reduction of the cross-sectional or throughput area. Preferably, by properly adjusting process parameters such as temperature and pressure, the resulting shear forces and shear velocities are of such magnitude, that the starch- and protein-containing feed ingredients become fluid.

"Increased pressure" means increased as compared to normal atmospheric pressure, and the maximum pressure as measured within the agglomeration unit.

The addition of water vapour or steam is often included in agglomeration, but not as an absolute requirement.

Agglomeration includes, but is not limited to, the well-known processes called extrusion, expansion (or pressure conditioning) and pelleting (or pellet pressing).

Extrusion is i.a. described at pp. 149-153 of a handbook which is available on request from the Danish Company Sprout-Matador, Glentevej 5-7, DK-6705 Esbjerg Ø or Niels Finsensvej 4, DK-7100 Vejle ("Håndbog i Pilleteringsteknik 1996"). However, in the agglomeration process of the invention, the following

process steps mentioned in the above handbook are entirely optional:

(i) pre-treating the feed ingredients in a cascade mixer;

(ii) cutting the product leaving the nozzle-section into pieces

(iii) of a desired size;

(iv) acclimatizing or conditioning it;

(v) coating it;

(vi) drying it;

(vii) cooling it.

The process of expansion (pressure conditioning) is i.a. described in the same handbook at pp. 61-66. Also for expansion, the above process steps (i)-(vi), in particular steps (i) and (vi), are entirely optional steps.

This is so also for the following process steps:

(ii') comminuting the product (using e.g. a blade granulator as shown at p. 65);

(vii) pelleting the product (using e.g. a pellet press as shown at p. 62);

The process of pelleting is i.a. described in the same handbook at pp. 71-107. Also here, steps (i)-(vii) above are entirely optional steps. These steps are i.a. described in more detail at pp. 29-70 of the above handbook.

In a preferred agglomeration process of the invention, one or more of the above mentioned further process steps (i)-(vii) are included.

A particularly preferred further step is step (i).

In a most preferred embodiment, the feed-ingredients are pre-heated in a first step (a) to a temperature of at least 45°C, preferably at least 50, 55, 60, 65, 70, 75, 80 °C; and then heated in a second step (b) to a temperature of at least

65°C, preferably 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at least 130°C.

The addition of thermostable phytase takes place before or during step (a) and/or before or during step (b).

Water is preferably added in step (a). More preferably, heated steam is added during the mixing of the ingredients (steps (a) and/or (b)).

Process step (a) is preferably performed in a cascade mixer (see the above cited handbook p. 44).

A "thermostable" phytase is a phytase which has a Tm (melting temperature) as measured on purified phytase protein by Differential Scanning Calorimetry (DSC) of at least 65°C, preferably using for the DSC a constant heating rate, more preferably of 10°C/min. In preferred embodiments, the Tm is at least 66, 67, 68, 69, 70, 71, 72, 73, 74 or 75°C. Preferably, the Tm is equal to or lower than 150°C, more preferably equal to or lower than 145, 140, 135, 130, 125, 120, 115 or 110°C. Accordingly, preferred intervals of Tm are: 65-150°C, 66-150°C, - (etc.) - 75-150°C; 65-145°C, 66-145°C, - (etc.) - 75-145°C; 65-140°C, - (etc.) - 75-140°C; - (etc.) - 65-110°C, 66-110°C, - (etc.) - 75-110°C.

Particularly preferred ranges for Tm are the following: between 65 and 110°C; between 70 and 110°C; between 70 and 100°C; between 75 and 95°C, or between 80 and 90°C.

In Example 3 below, the measurement of Tm by DSC is described, and the Tm's of a number of phytases are shown.

The optimum temperatures are also indicated, since - in the alternative - a thermostable phytase can be defined as a phytase having a temperature-optimum of at least 60°C. Preferably, the optimum temperature is determined on the substrate phytate at pH 5.5, or on the substrate phytic acid at

pH 5.0. Preferred units are FYT, FTU or the units of Example 3. The phytase assay of Example 3 is most preferred.

In preferred embodiments, the optimum temperature is at least 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70°C. Preferably, the optimum temperature is equal to or lower than 140°C, more preferably equal to or lower than 135, 130, 125, 120, 115, 110, 105 or 100°C. Accordingly, preferred intervals of optimum temperature are: 60-140°C, 61-140°C, - (etc.) - 70-140°C; 60-135°C, 61-135°C, - (etc.) - 70-135°C; 60-130°C, - (etc.) - 70-130°C; - (etc.) - 60-100°C, 61-100°C, - (etc.) - 70-100°C.

Preferred phytases of the present invention exhibit a degree of similarity or homology, preferably identity, to the complete amino acid sequence of either of the phytases mentioned below under (iii) - preferably to the complete amino acid sequence of Consensus-phytase-10-thermo-Q50T-K91A - of at least 48%, preferably at least 50, 52, 55, 60, 62, 65, 67, 70, 73, 75, 77, 80, 82, 85, 88, 90, 92, 95, 98 or 99%.

The degree of similarity or homology, alternatively identity, can be determined using any alignment programme known in the art. A preferred alignment programme is GAP provided in the GCG version 8 program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (see also Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453). Using GAP with the following settings for polypeptide sequence comparison: GAP weight of 3.000 and GAP lengthweight of 0.100.

A multiple sequence alignment can be made using the program PileUp (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science

Drive, Madison, Wisconsin, USA 53711), with a GapWeight of 3.000 and a GapLengthWeight of 0.100.

Using the program GAP, some selected phytases exhibit the following percentage similarity (identity in brackets) to the Consensus-phytase-10-thermo(3)-Q50T-K91A amino acid sequence:

A. fumigatus ATCC-13073 α-mutant	86.7% (81.8%)
Basidiomycet consensus	64.1% (49.0%)
Consensus-phytase-1	98.7% (97.9%)
Consensus-phytase-10	96.6% (94.4%)
Consensus-phytase-1-thermo(8)-Q50T-K91A	97.4% (95.5%)
Consensus-phytase-11	96.5% (94.2%)
Consensus-phytase-12	92.5% (89.9%)
Consensus-phytase-7	95.5% (93.4%)

A "purified" phytase is essentially free of other non-phytase polypeptides, e.g. at least about 20% pure, preferably at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by SDS-PAGE.

Preferred thermostable phytases are the so-called consensus phytases of EP 98113176.6 (EP 0897985), viz.

(i) any thermostable phytase which is obtainable by the processes described therein;

(ii) a phytase comprising the amino acid sequence shown in Fig. 2 thereof or any variant or mutein thereof, preferred muteins being those comprising the substitutions Q50L; Q50T; Q50G; Q50T-Y51N or Q50L-Y51N.

Other preferred thermostable phytases are

(iii) a thermostable phytase which comprises at least one of the following amino acid sequence (some of which are shown in Figs. 5-12 herein), preferably the following phytases: Consensus-phytase-1 (or simply Consensus phytase); Consensus-phytase-1-thermo(3); Consensus-phytase-1-Q50T; basidiomycete-consensus (or simply Basidio); Consensus-phytase-10 (or Fcp 10); Consensus-phytase-11 (or Consensus Seq. 11); Consensus-phytase-1-thermo(8)-Q50T-K91A; Consensus-phytase-1-thermo(8)-Q50T; Consensus-phytase-1-thermo(8); Consensus-phytase-10-thermo(3)-Q50T-K91A; Consensus-phytase-10-thermo(3)-Q50T (sometimes, "(3)" is deleted from this expression); Aspergillus fumigatus ATCC 13073 phytase α-mutant; Aspergillus fumigatus ATCC 13073 phytase α-mutant plus the mutations E59A, S126N, R329H, S364T, G404A; Aspergillus fumigatus ATCC 13073 phytase α-mutant plus the mutations E59A, K68A, S126N, R329H, S364T, G404A; Consensus-phytase-7; Consensus-phytase-12.

(iv) as well as thermostable variants and muteins of the phytases of (iv) and (v), in particular those comprising one or more of the following substitutions: Q50L,T,G; Q50L-Y51N; Q50T-Y51N.

The term "plant" is intended to include not only whole plants as such, but also plant parts or organs, such as leaves, seeds or grains, stem, root, tubers, flowers, callus, fruits etc.; tissues, cells, protoplats etc.; as well as any combinations or sub-combinations thereof. Plant tissue cultures and plant cell lines as well as plant protoplasts are specifically included herein.

The term "transgenic plant" is a plant as defined above, which has been genetically modified, as well as its progeny and propagating material thereof having retained the genetical modification. Preferably, the transgenic plant comprises at least one specific gene introduced into an ancestral plant by recombinant gene technology. The term is not confined to a single plant variety.

The invention relates to a transgenic plant which comprises a DNA-construct encoding a thermostable phytase.

In a preferred embodiment the transgenic plant is a plant grouping which is characterized in that it comprises a DNA-construct encoding a thermostable phytase. The members of this plant grouping may very well possess individuality, but are clearly distinguishable from other varieties by their common characteristic feature of the the thermostable phytase DNA-construct.

Accordingly, the present teaching is applicable to more than one plant variety. No naturally occuring plant varieties are included amongst the plants of the invention.

In another preferred embodiment the invention relates to a transgenic plant variety or a variant thereof; a transgenic plant species, a transgenic plant genus, a transgenic plant family, and/or a transgenic plant order. More preferably, plant varieties as such are disclaimed.

Any thermostable phytase may be used in the present invention, e.g. any wild-type phytases, genetically engineered phytases, consensus phytases, phytase muteins, and/or phytase variants. Genetically engineered phytases include, but are not limited to, phytases prepared by site-directed mutagenesis, gene shuffling, random mutagenesis, etc.

The nucleotide sequence encoding a wild-type thermostable phytase may be of any origin, including mammalian, plant and microbial origin and may be isolated from these sources by conventional methods. Preferably, the nucleotide sequence is derived from a microorganism, such as a fungus, e.g. a yeast or a filamentous fungus, or a bacterium. The DNA sequence encoding a thermostable phytase may be isolated from the cell producing it, using various methods well known in the art (see e.g. WO 98/28409 and EP 0897985).

The nucleotide sequence encoding a thermostable genetically engineered or consensus phytase, including muteins and variants thereof, may be prepared in any way, e.g. as described in Example 3 hereof and in EP 0897985.

In order to accomplish expression of the thermostable phytase in a plant of the invention the nucleotide sequence encoding the phytase is inserted into an expression construct containing regulatory elements or sequences capable of directing the expression of the nucleotide sequence and, if necessary or desired, to direct secretion of the gene product or targetting of the gene product to the seeds of the plant.

In order for transcription to occur the nucleotide sequence encoding the thermostable phytase is operably linked to a suitable promoter capable of mediating transcription in the plant in question. The promoter may be an inducible promoter or a constitutive promoter. Typically, an inducible promoter mediates transcription in a tissue-specific or growth-stage specific manner, whereas a constitutive promoter provides for sustained transcription in all cell tissues. An example of a suitable constitutive promoter useful for the present invention is the cauliflower mosaic virus 35 S promoter. Transcription initiation sequences from the tumor-inducing plasmid (Ti) of

Agrobacterium such as the octopine synthase, nopaline synthase, or mannopine synthase initiator, are further examples of preferred constitutive promoters.

Examples of suitable inducible promoters include a seed-specific promoter such as the promoter expressing alpha-amylase in wheat seeds (see Stefanov et al, Acta Biologica Hungarica. Vol. 42, No. 4 pp. 323-330 (1991), a promoter of the gene encoding a rice seed storage protein such as glutelin, prolamin, globulin or albumin (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a Vicia faba promoter from the legumin B4 and the unknown seed protein gene from Vicia faba described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), the storage protein napA promoter from Brassica napus, or any other seed specific promoter known in the art, eg as described in WO 91/14772.

In order to increase the expression of the thermostable phytase it is desirable that a promoter enhancer element is used. For instance, the promoter enhancer may be an intron which is placed between the promoter and the amylase gene. The intron may be one derived from a monocot or a dicot. For instance, the intron may be the first intron from the rice Waxy (Wx) gene (Li et al., Plant Science Vol. 108, No. 2, pp. 181-190 (1995)), the first intron from the maize Ubi1 (Ubiquitin) gene (Vain et al., Plant Cell Reports Vol. 15, No. 7 pp. 489-494 (1996)) or the first intron from the Act1 (actin) gene. As an example of a dicot intron the chsA intron (Vain et al. op cit.) is mentioned. Also, a seed specific enhancer may be used for increasing the expression of the thermostable phytase in seeds. An example of a seed specific enhancer is the one derived from the beta-phaseolin gene encoding the major seed storage protein of bean

(Phaseolus vulgaris) disclosed by Vandergeest and Hall, Plant Molecular Biology Vol. 32, No. 4, pp. 579-588 (1996).

Also, the expression construct preferably contains a terminator sequence to signal transcription termination of the thermostable phytase gene such as the rbcS2' and the nos3' terminators.

To facilitate selection of successfully transformed plants, the expression construct should also preferably include one or more selectable markers, e.g. an antibiotic resistance selection marker or a selection marker providing resistance to a herbicide. One widely used selection marker is the neomycin phosphotransferase gene (NPTII) which provides kanamycin resistance. Examples of other suitable markers include a marker providing a measurable enzyme activity, e.g. dihydrofolate reductase, luciferase, and b-glucoronidase (GUS). Phosphinothricin acetyl transferase may be used as a selection marker in combination with the herbicide basta or bialaphos.

The transgenic plant of the invention may be prepared by methods known in the art. The transformation method used will depend on the plant species to be transformed and can be selected from any of the transformation methods known in the art such as Agrobacterium mediated transformation (Zambryski et al., EMBO Journal 2, pp 2143-2150, 1993), particle bombardment, electroporation (Fromm et al. 1986, Nature 319, pp 791-793), and virus mediated transformation. For transformation of monocots particle bombardment (ie biolistic transformation) of embryogenic cell lines or cultured embryos are preferred. Below, references are listed, which disclose various methods for transforming various plants: Rice (Cristou et al. 1991, Bio/Technology 9, pp. 957-962), Maize (Gordon-Kamm et al. 1990, Plant Cell 2, pp. 603-618), Oat (Somers et al. 1992,

Bio/Technology 10, pp 1589-1594), Wheat (Vasil et al. 1991, Bio/Technology 10, pp. 667-674, Weeks et al. 1993, Plant Physiology 102, pp. 1077-1084) and Barley (Wan and Lemaux 1994, Plant Physiology 102, pp. 37-48, review Vasil 1994, Plant Mol. Biol. 25, pp 925-937).

More specifically, Agrobacterium mediated transformation is conveniently achieved as follows:

A vector system carrying the thermostable phytase is constructed. The vector system may comprise of one vector, but it can comprise of two vectors. In the case of two vectors the vector system is referred to as a binary vector system (Gynheung An et al.(1980), Binary Vectors, Plant Molecular Biology Manual A3, 1-19).

An Agrobacterium based plant transformation vector consists of replication origin(s) for both E.coli and Agrobacterium and a bacterial selection marker. A right and preferably also a left border from the Ti plasmid from Agrobacterium tumefaciens or from the Ri plasmid from Agrobacterium rhizogenes is nessesary for the transformation of the plant. Between the borders the expression construct is placed which contains the thermostable phytase gene and appropriate regulatory sequences such as promotor and terminator sequences. Additionally, a selection gene e.g. the neomycin phosphotransferase type II (NPTII) gene from transposon Tn5 and a reporter gene such as the GUS (betha-glucuronidase) gene is cloned between the borders. A disarmed Agrobacterium strain harboring a helper plasmid containing the virulens genes is transformed with the above vector. The transformed Agrobacterium strain is then used for plant transformation.

The invention also relates to a method of preparing a transgenic plant capable of expressing a thermostable phytase,

said method comprising the steps of (i) isolating a nucleotide sequence encoding a thermostable phytase; (ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and (iii) transforming the selected host plant with the expression construct.

The above method in which "at least one" replaces "a," when used in relation to the thermostable phytase, is also within this invention.

This method is an essentially non-biological method.

Any plant may be a selected host plant. More specifically, the plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Of primary interest are such plants which are potential food or feed components. These plants may comprise phytic acid. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley, rice, sorghum and maize (corn).

Examples of dicot plants are legumes, such as lupins, pea, bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis thaliana.

Of particular interest are monocotyledonous plants, in particular crops or cereal plants such as wheat (Triticum, e.g. aestivum), barley (Hardeum, e.g. vulgare), oats, rye, rice, sorghum and corn (Zea, e.g. mays).

Of further particular interest are dicotyledonous plants, such as those mentioned above.

In a preferred embodiment, the ancestral plant or host plant is per se a desired feed ingredient.

Examples

Example 1

FYT-assay - for analyzing phytase enzyme preparations

The phytase activity can be measured using the following assay: 10 µl diluted enzyme samples (diluted in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5) are added into 250 µl 5 mM sodium phytate (Sigma) in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5 (pH adjusted after dissolving the sodium phytate; the substrate is preheated) and incubated for 30 minutes at 37°C. The reaction is stopped by adding 250 µl 10 % TCA and free phosphate is measured by adding 500 µl 7.3 g FeSO4 in 100 ml molybdate reagent (2.5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 8 ml H_2SO_4 diluted to 250 ml). The absorbance at 750 nm is measured on 200 µl samples in 96 well microtiter plates. Substrate and enzyme blanks are included. A phosphate standard curve is also included (0-2 mM phosphate). 1 FYT equals the amount of enzyme that releases 1 µmol phosphate/min at the given conditions. This assay is preferred for phytase enzyme preparations (when not in admixture with other feed ingredients).

Example 2

FTU assay - for analyzing phytase in admixture with feed ingredients

One FTU is defined as the amount of enzym, which at standard conditions (37°C, pH 5,5; reaction time 60 minutes and start concentration of phytic acid 5 mM) releases phosphate equivalent to 1 µmol phosphate per minute.

1 FTU = 1 FYT

The FTU assay is preferred for phytase activity measurements on animal feed premixes and the like complex compositions.

Reagents /substrates

Extraction buffer for feed etc.

This buffer is also used for preparation of PO_4-standards and further dilution of premix samples.

0,22 M acetate buffer with Tween 20 pH 5,5

30 g sodium acetate trihydrate (MW = 136,08 g/mol) e.g. Merck Art 46267 per liter and 0,1 g Tween 20 e.g. Merck Art 22184 pr. liter are weighed out.

The sodium acetate is dissolved in demineralised water.

Tween 20 is added, and pH adjusted to 5,50 ± 0,05 with acetic acid.

Add demineralised water to total volume.

Extraction buffer for premix

0,22 M acetate buffer with Tween 20, EDTA, PO_4^{3-} og BSA.

30 g sodium acetate trihydrate e.g. Merck Art 6267 per liter.

0,1 g Tween 20 e.g. Merck Art 22184 per liter.

30 g EDTA f.eks. Merck Art 8418 pr. liter.

20 g Na_2HPO_4, $2H_2O$ e.g. Merck Art 6580 per liter.

0,5 g BSA (Bovine Serum Albumine, e.g. Sigma Art A-9647 per liter.

The ingredients are dissolved in demineralised water, and pH is adjusted to 5,50 ± 0,05 with acetic acid.

Add demineralised water to total volume.

BSA is not stable, and must therefore be added the same day the buffer is used.

50 mM PO_4^{3-} stock solution

0,681 g KH2PO4 (MW = 136,09 g/mol) e.g. Merck Art 4873 is weighed out and dissolved in 100 ml 0,22 M sodium acetat with Tween, pH 5,5.

Storage stability: 1 week in refrigerator.

0,22 M acetate buffer pH 5,5 without Tween

This buffer is used for production of phytic acid substrate).

150 g sodium acetate trihydrate (MW = 136,08) e.g. Merck Art 6267 is weighed out and dissolved in demineralised water, and pH is adjusted with acetic acid to 5,50 ± 0,05.

Add demineralised water to 5000 ml.

Storage stability: 1 week at room temperature.

Phytic acid substrate; 5 mM phytic acid

The volume of phytic acid is calculated with allowance for the water content of the used batch.

If the water content is e.g. 8,4 % the following is obtained:

$$\frac{0{,}005\,mol/l \times 923{,}8\,g/mol}{(1 \div 0{,}084)} = 5{,}04\,g/l$$

Phytic acid (Na-salt) (MW = 923,8 g/mol) e.g. Sigma P-8810 is weighed out and dissolved in 0,22 M acetate buffer (without tween). Addition of (diluted) acetic acid increases the dissolution speed.

pH is adjusted to 5,50 ± 0,05 with acetic acid.

Add 0,22 M acetate buffer to total volume.

21,7 % nitric acid solution

For stop solution.

1 part concentrated (65%) nitric acid is mixed into 2 parts demineralised water.

Molybdate reagent

For stop solution.

100 g ammonium heptamolybdate tetrahydrate $(NH_4)6Mo_7O_{24},4H_2O$ e.g. Merck Art 1182 is dissolved in demineralised water. 10 ml 25 % NH_3 is added.

Add demineralised water to 1 liter.

0,24 % Ammonium vanadate

Bought from fra Bie & Berntsen.

Molybdat/vanadat stop solution

1 part vanadate solution (0,24 % ammonium vanadate) + 1 part molybdate solution are mixed. 2 parts 21,7 % nitric acid solution are added.

The solution is prepared not more than 2 hours before use, and the bottle is wrapped in tinfoil.

Samples

Frozen samples are defrosted in a refrigerator overnight.

Sample size for feed samples: At least 70 g, preferably 100 g.

Feed samples

Choose a solution volume which allows addition of buffer corresponding to 10 times the sample weight, e.g. 100 g is dissolved in 1000 ml 0,22 M acetate buffer with Tween, see enclosure 1. Round up to nearest solution volume.

If the sample size is approx. 100 g all the sample is ground in a coffee grinder and subsequently placed in tared

beakers. The sample weight is noted. It is not necessary to grind not-pelleted samples. If a sample is too big to handle, it is sample split into parts of approx. 100 g.

Magnets are placed in the beakers and 0,22 M acetate buffer with Tween is added.

The samples are extracted for 90 minutes.

After extraction the samples rest for 30 minuts to allow for the feed to sediment. A 5 ml sample is withdrawn with a pipette. The sample is taken 2 - 5 cm under the surface of the solution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

<u>Premix samples</u>

Choose a solution volume which allows addition of buffer corresponding to 10 times the sample weight. Round up to nearest solution volume.

If the samples have been weighed (50 - 100 g) all of the sample is placed in tared beakers. The sample weight is noted. If a sample is too big to handle, it is split into parts of approx. 100 g.

Magnets are placed in the beakers and 0,22 M acetate buffer with Tween, EDTA og PO_4^{3-} is added.

The samples are extracted for 60 minutes.

After extraction the samples rest for 30 minutes to allow for the premix to sediment. A 5 ml sample is withdrawn with a pipette. The sample is taken 2 - 5 cm under the surface of the solution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

Analysis

Extracts of feed samples are analysed directly.

Extracts of premix are diluted to approx. 1,5 FTU/g (A_{415} (main sample) < 1,0).

0,22 M acetate buffer with Tween 20 is used for the dilution.

Main Samples

2 x 100 ml of the supernatant from the extracted and centrifuged samples are placed in marked glass test tubes and a magnet is placed in each tube.

When all samples are ready they are placed on a water bath with stirring. Temperature: 37 °C.

3,0 ml substrate is added.

Incubation for exactly 60 minutes after addition of substrate.

The samples are taken off the water bath and 2,0 ml stop solution is added (exactly 60 minutes after addition of substrate).

The samples are stirred for 1 minute or longer.

Feed samples are centrifuged for 10 minutes at 4000 rpm (It is not necessary to centrifuge premix samples).

Blind samples

100 ml of the supernatant from the extracted and centrifuged samples are placed in marked glass test tubes, and a magnet is placed in each tube.

2,0 ml stop solution is added to the samples.

3,0 ml substrate is added to the samples.

The samples are incubated for 60 minutes at room temperature.

The feed samples are centrifuged for 10 minutes at 4000 rpm (it is not necessary to centrifuge premix samples).

Standards

2 x 100 ml are taken from each of the 8 standards and also 4 x 100 ml 0,22 M acetate buffer (reagent blind).

A_{415} is measured on all samples.

CALCULATION

FTU/g = μmol PO_4^{3-} / (min * g (sample))

C g sample is weighed out (after grinding).

100 μl is taken from the extracted and centrifuged sample.

PO_4^{3-} standard curve is linear.

From the regression curve for the PO_4^{3-} standard the actual concentration of the sample is found (concentration in mM):

$[PO_4^{3-}] = (x - b) / a$ $\quad x = A_{415}$ $\quad a = slope$ $\quad b =$ *intercept with y-axis*

μmol PO_4^{3-}/min = { $[PO_4^{3-}]$ (mM) × Vol (liter) × 1000 μmol/mmol } /t

t = incubation time in minutes.

Vol = sample volume in liter = 0,0001 liter

1000 = conversion factor from mmol to μmol

$$\text{FTU } /g_{prøve} = \{ (x - b) \times Vol \times 1000 \times F_p\} / \{ a \times t \times C\}$$

C = gram sample weighed out

F_p = Relation between the sample taken out and the total sample (after extraction). Example: 0,100 ml taken from 1000 ml $\rightarrow F_p$ = 1000/0,100 = 10000.

Reduced expression with insertion of the following values:

t = 60

Vol = 0,0001 l

F_p = 10000

$$\text{FTU } /g_{sample} = \{ (x - b) \times 0{,}0001 \times 1000 \times 10000\} / \{ a \times 60 \times C\}$$

Example 3

Determination of optimum temperature and melting point Tm of various phytases

The thermostability of various phytases has been determined, viz. the melting temperature, Tm, and/or the optimum temperature.

The phytase of Aspergillus niger NRRL 3135 was prepared as described in EP 0420358 and van Hartingsveldt et al (Gene, 127, 87-94, 1993).

The phytases of Aspergillus fumigatus ATCC 13073, Aspergillus terreus 9A-1, Aspergillus terreus CBS 116.46, Aspergillus nidulans, Myceliophthora thermophila, and Talaromyces thermophilus were prepared as described in EP-0897985 and the references therein.

Consensus-phytase-1 (Fig. 5) and Consensus-phytase-1-Q50T are shown in and were prepared as described in EP 0897985.

Consensus-phytase-10 was derived and prepared according to the teachings of EP-0897985 (Examples 1-2 and 3-7, respectively), however adding to the alignment at Fig. 1 thereof the phytase sequence of Thermomyces lanuginosa (Berka et al, Appl. Environ. Microbiol. 64, 4423-4427, 1998) and a basidiomycete consensus sequence (derivation described below), omitting the sequence of A.niger T213, and assigning a vote weight of 0.5 for the remaining A.niger phytase sequences. The derivation of the sequence of Consensus-phytase-10 is shown in Fig. 7.

The basidiomycete consensus sequence was also derived according to the principles of EP-0897985, viz. from the five basidiomycete phytases of WO 98/28409, starting with the first amino acid residue of the mature phytases (excluding signal peptide). A vote weight of 0.5 was assigned to the two Paxillus phytases, all other genes were used with a vote weight of 1.0 - see Fig. 6.

The muteins Consensus-phytase-10-thermo, Consensus-phytase-10-thermo-Q50T-K91A (Fig. 10) and Consensus-phytase-10-thermo-Q50T were prepared from consensus-phytase-10, in analogy to Examples 5-8 of EP-0897985, by introducing the three back-mutations K94A, V158I and A396S ("thermo(3)" or "thermo") and, where applicable, also the mutations Q50T or Q50T-K91A.

The muteins Consensus-phytase-1-thermo(8), Consensus-phytase-1-thermo(8)-Q50T-K91A (Fig. 9) and Consensus-phytase-1-thermo(8)-Q50T, were prepared from consensus-phytase-1, in analogy to Example 8 of EP-0897985, by introducing the eight mutations E58A, D197N, E267D, R291I, R329H, S364T, A379K and G404A ("thermo(8)") and, where applicable, also the mutations Q50T or Q50T-K91A.

Consensus-phytase-1-thermo(3) was prepared from consensus-phytase-1 by introduction of the three mutations K94A, V158I and A396S.

An Aspergillus fumigatus so-called α-mutant (with the mutations Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D) and the further muteins thereof shown in Table 1 were prepared as generally described above. The position numbering refers to Fig. 11 hereof, except for the number in parentheses which refers to the numbering used in EP 0897010.

DNA constructs encoding the above thermostable phytases can be prepared e.g. according to the teachings of EP 0897985. For expression thereof in plants, reference is made to the present description.

In order to determine the unfolding temperature or melting temperature, Tm, of a phytase, differential scanning calorimetry was applied as previously published by Brugger et al (1997): "Thermal denaturation of phytases and pH 2.5 acid phosphatase studied by differential scanning calorimetry," in The Biochemistry of phytate and phytase (eds. Rasmussen, S.K; Raboy, V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers).

Homogenous or purified phytase solutions of 50-60 mg/ml of protein are prepared, and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min is applied up to 90-95°C.

The results of Tm determinations on the above phytases are shown in Table 1 below; for selected phytases also in Figs. 1-4.

In Table 1 below, the optimum temperature of various phytases is also indicated. For this determination, phytase activity was determined basically as described by Mitchell et al (Microbiology 143, 245-252, 1997): The activity was measured in an assay mixture containing 0.5% phytic acid (~ 5 mM) in 200 mM

sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 µl of the assay mixture with 900µl H_2O and 1 ml of 0.6 M H_2SO_4, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al (Prot.Sci. 4, 2411-2423, 1995): Consensus phytase, 1.101; consensus phytase 7, 1.068; consensus phytase 10, 1.039.

For determination of the temperature optimum, enzyme (100µl) and substrate solution (100µl) were pre-incubated for 5 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined. Phytase-activity-versus-temperature is plotted, and the temperature optimum is determined as that temperature at which the acitivity reaches its maximum value.

Table 1

Temperature optimum and Tm for various phytases

Phytase	**Optimum temperature (°C)**	**Tm (°C)**
Aspergillus niger NRRL 3135	55	63.3
Aspergillus fumigatus ATCC 13073	55	62.5

Aspergillus terreus 9A-1	49	57.5
Aspergillus terreus CBS 116.46	45	58.5
Aspergillus nidulans	45	55.7
Myceliophthora thermophila	55	-
Talaromyces thermophilus	45	-
Consensus-phytase-10-thermo-Q50T-K91A	82	89.3
Consensus-phytase-10-thermo-Q50T	82	88.6
Consensus-phytase-10	80	85.4
Consensus-phytase-1-thermo(8)-Q50T-K91A	-	85.7
Consensus-phytase-1-thermo(8)-Q50T	78	84.7
Consensus-phytase-1-thermo(8)	81	-
Consensus-phytase-1-thermo(8)-Q50T-K91A	78	84.7
Consensus-phytase-1-thermo(3)	75	-
Consensus-phytase-1-Q50T	-	78.9
Consensus-phytase-1	71	78.1
Aspergillus fumigatus α-mutant, plus mutations E59A,	63	-

S126N, R329H, S364T, G404A		
Aspergillus fumigatus - as above, plus mutation K68A	63	-
Aspergillus fumigatus α-mutant (Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D)	60	67.0

CLAIMS

1. A process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the agglomeration.

2. The process of claim 1, wherein the feed ingredients are heated to a temperature of at least 65°C.

3. The process of any of claims 1-2, wherein the thermostable phytase is a phytase with a Tm as measured by DSC of at least 65°C, using for the DSC a constant heating rate of 10°C/min.

4. The process of any of claims 1-3, when performed in a feed expander.

5. The process of any of claims 1-3, when performed in an extruder.

6. The process of any of claims 1-3, when performed in a pellet press.

7. The process of any of claims 1-6, wherein the thermostable phytase is present in a transgenic plant.

8. The process of any of claims 1-7, wherein the agglomeration includes the following steps:

(a) pre-heating the feed ingredients to a temperature of at least 45°C; and

(b) heating the product of step (a) to a temperature of at least 65°C;

wherein the thermostable phytase is added prior to or during step (a) and/or (b).

9. A transgenic plant which comprises a DNA-construct encoding a thermostable phytase.

10. The transgenic plant of claim 9, wherein the DNA-construct encoding the thermostable phytase is operably linked to regulatory sequences capable of mediating expression of said phytase encoding sequence in at least one part of the plant.

11. An expression construct which comprises a DNA construct encoding a thermostable phytase, operably linked to regulatory sequences capable of mediating expression of said phytase encoding sequence in at least one part of a plant.

12. A vector which comprises the expression construct of claim 11.

13. A method of preparing a transgenic plant capable of expressing a thermostable phytase, said method comprising the steps of
(i) isolating a nucleotide sequence encoding a thermostable phytase;
(ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and
(iii) transforming the selected host plant with the expression construct.

14. The method of claim 13, which comprises the further step of extracting the phytase from the plant.

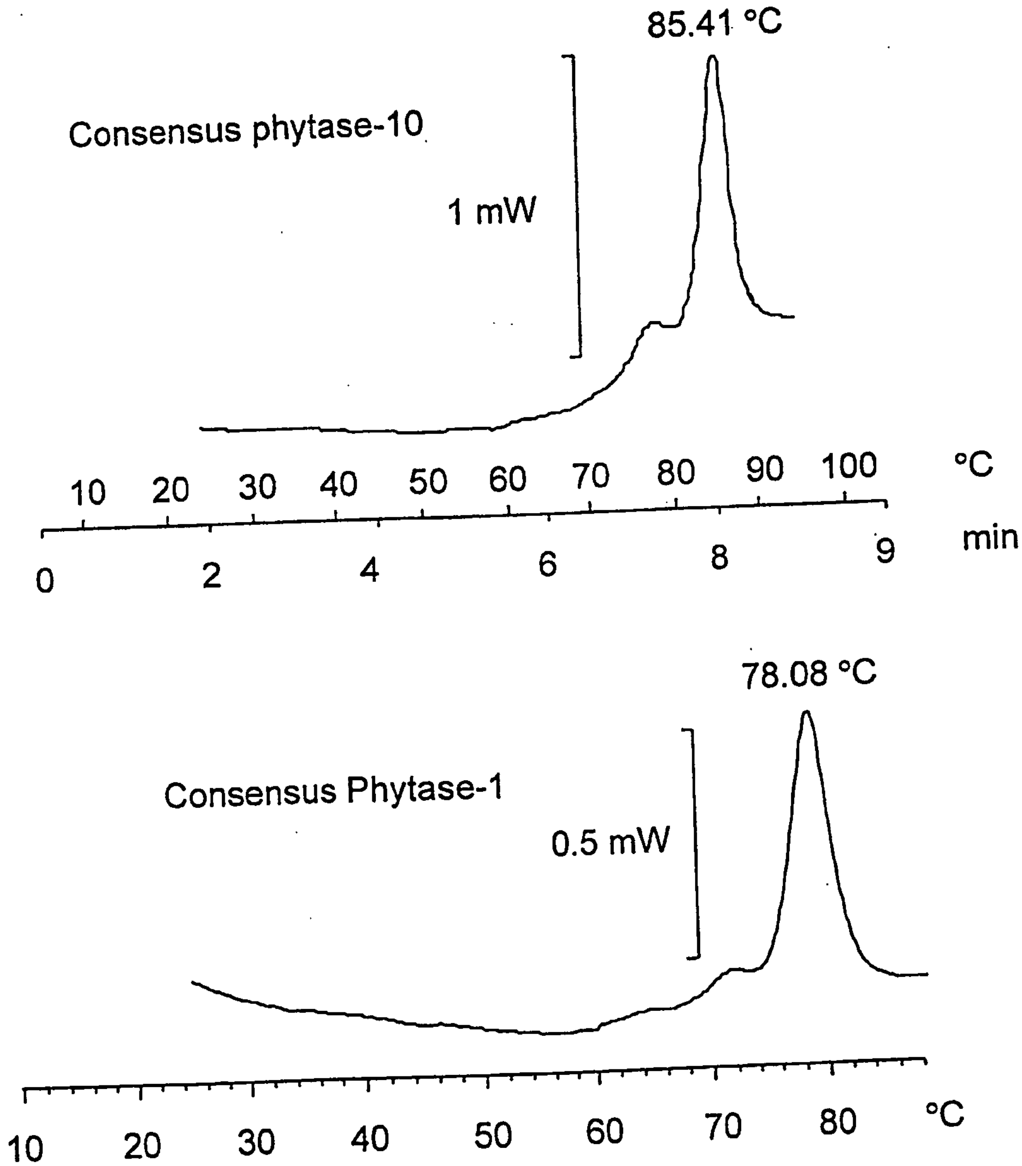
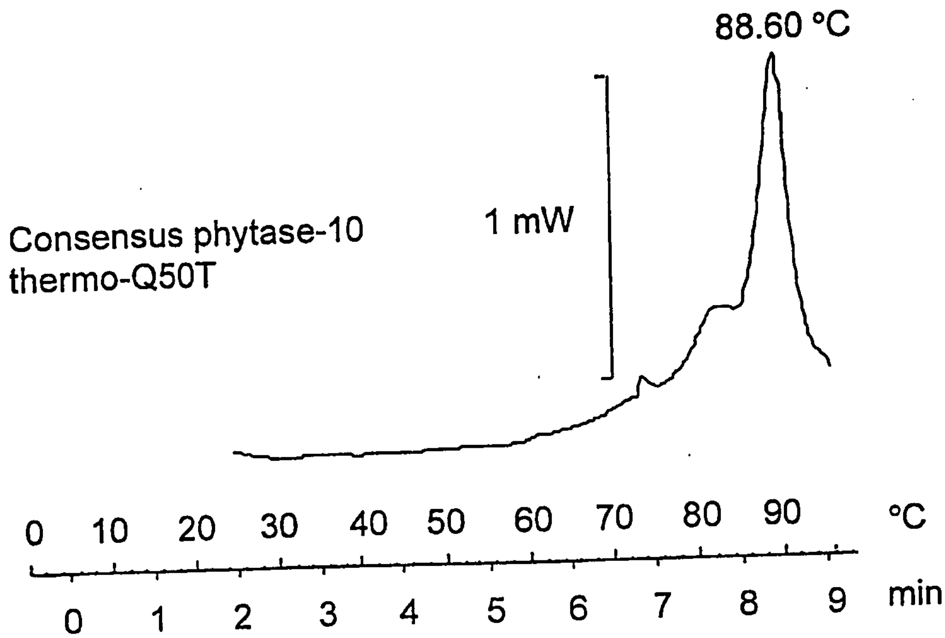

85.41 °C
Consensus phytase-10
1 mW
10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min
78.08 °C
Consensus Phytase-1
0.5 mW
10 20 30 40 50 60 70 80 °C

Fig. 1


88.60 °C
Consensus phytase-10
thermo-Q50T
1 mW
0 10 20 30 40 50 60 70 80 90 °C
0 1 2 3 4 5 6 7 8 9 min

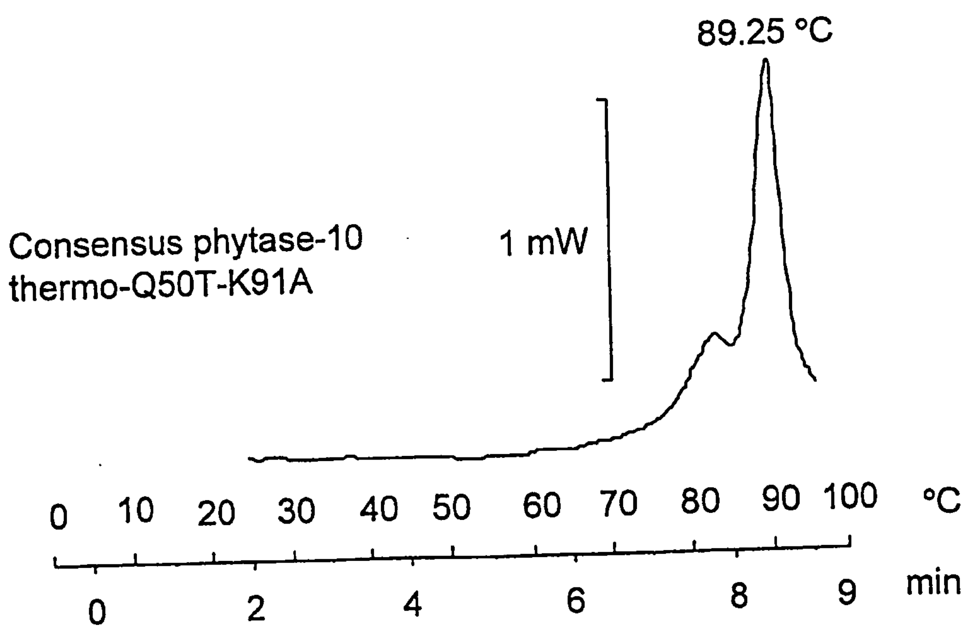
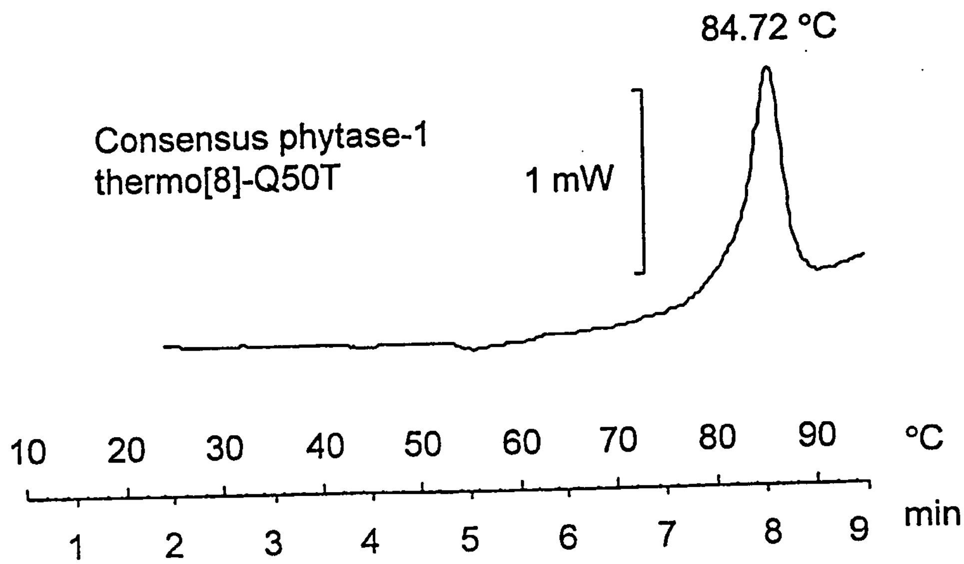

89.25 °C
Consensus phytase-10
thermo-Q50T-K91A
1 mW
0 10 20 30 40 50 60 70 80 90 100 °C
0 2 4 6 8 9 min

Fig. 2


84.72 °C
Consensus phytase-1
thermo[8]-Q50T
1 mW
10 20 30 40 50 60 70 80 90 °C
1 2 3 4 5 6 7 8 9 min

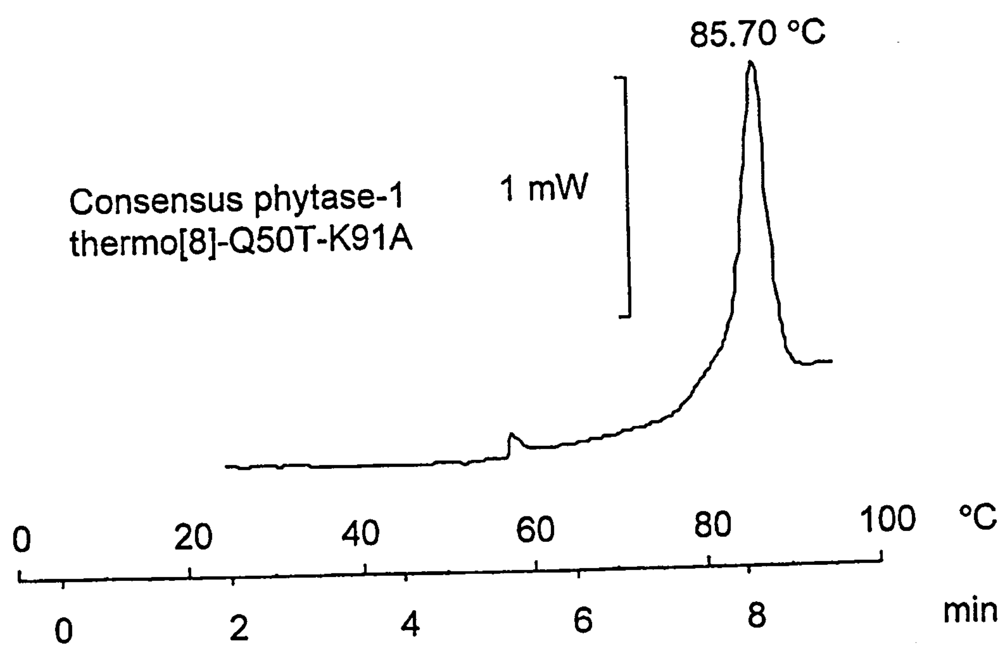

85.70 °C
Consensus phytase-1
thermo[8]-Q50T-K91A
1 mW
0 20 40 60 80 100 °C
0 2 4 6 8 min

Fig. 3

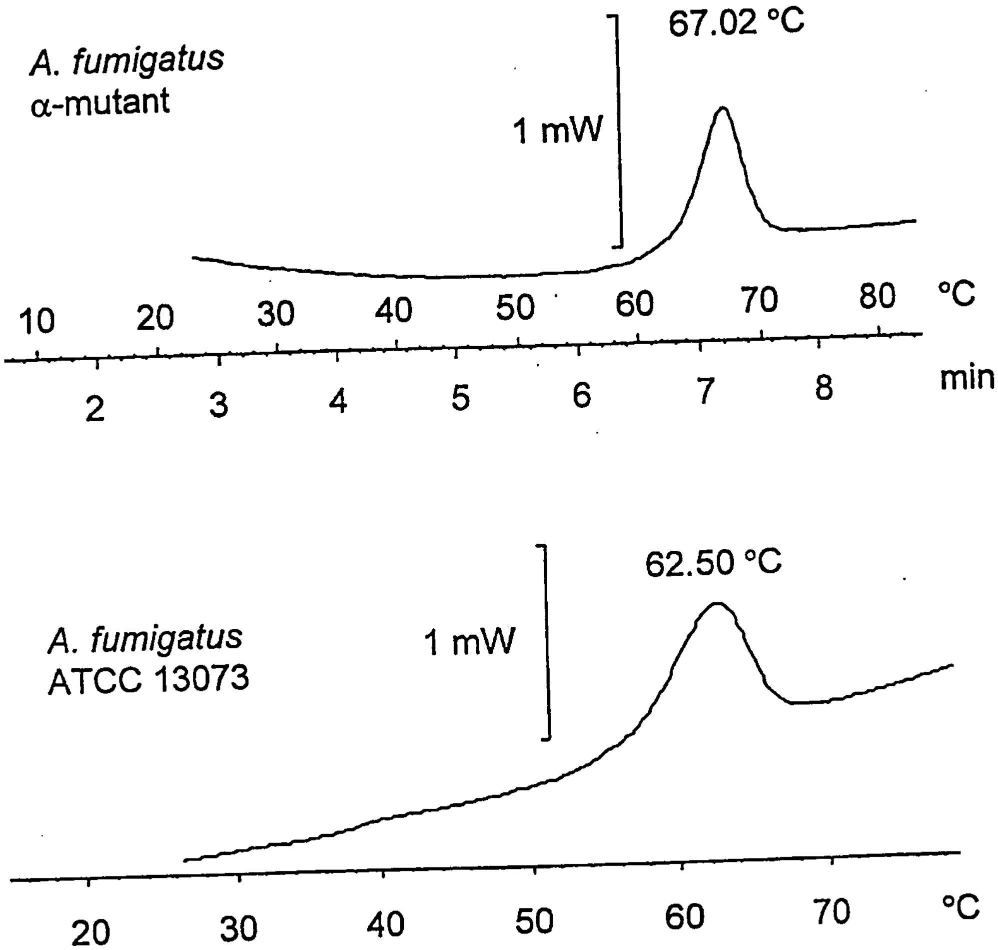

A. fumigatus
α-mutant
1 mW
67.02 °C
10 20 30 40 50 60 70 80 °C
2 3 4 5 6 7 8 min
A. fumigatus
ATCC 13073
1 mW
62.50 °C
20 30 40 50 60 70 °C

Fig. 4

	1				50
A. *terreus* 9A-1	KhsDCNSVDh	GYQCFPELSH	kWGlYAPYFS	LQDESPFPlD	VPEDChITFV
A. *terreus* cbs	NhsDCTSVDr	GYQCFPELSH	kWGlYAPYFS	LQDESPFPlD	VPDDChITFV
A. *niger* var. *awamori*	NqsTCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESAISPD	VPAGCrVTFA
A. *niger* T213	NqsSCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPD	VPAGCrVTFA
A. *niger* NRRL3135	NqsSCDTVDQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPE	VPAGCrVTFA
A. *fumigatus* 13073	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. *fumigatus* 32722	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. *fumigatus* 58128	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. *fumigatus* 26906	GSkSCDTVDl	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
A. *fumigatus* 32239	GSkACDTVEl	GYQCsPGTSH	LWGQYSPFFS	LEDElSVSSD	LPKDCrVTFV
E. *nidulans*	QNHSCNTADG	GYQCFPNVSH	VWGQYSPYFS	IEQESAISeD	VPHGCeVTFV
T. *thermophilus*	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCkITFV
M. *thermophila*	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VpSElDaS..	IPDDCeVTFA
Consensus	NSHSCDTVDG	GYQCFPEISH	LWGQYSPYFS	LEDESAISPD	VPDDC-VTFV
Consensus phytase	NSHSCDTVDG	GYQCFPEISH	LWGQYSPYFS	LEDESAISPD	VPDDCRVTFV

	51				100
A. *terreus* 9A-1	QVLARHGARs	PThSKtKAYA	AtIAAIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. *terreus* cbs	QVLARHGARs	PTDSKtKAYA	AtIAAIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. *niger* var. *awamori*	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
A. *niger* T213	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
A. *niger* NRRL3135	QVLSRHGARY	PTDSKgKkYS	ALIEEIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. *fumigatus* 13073	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* 32722	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* 58128	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* 26906	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* 32239	QVLSRHGARY	PTASKsKkYK	kLVTAIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. *nidulans*	QVLSRHGARY	PTESKsKAYS	GLIEAIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. *thermophilus*	QLLSRHGARY	PTSSKtElYS	QLISrIQKTA	TaYKGyYAFL	KDYrYqLGAN
M. *thermophila*	QVLSRHGARa	PTlKRaaSYv	DLIDrIHhGA	IsYgPgYEFL	RTYDYTLGAD
Consensus	QVLSRHGARY	PTSSK-KAYS	ALIEAIQKNA	T-FKGKYAFL	KTYNYTLGAD
Consensus phytase	QVLSRHGARY	PTSSKSKAYS	ALIEAIQKNA	TAFKGKYAFL	KTYNYTLGAD

	101				150
A. *terreus* 9A-1	ELTPFGrNQL	rDlGaQFYeR	YNALTRhInP	FVRATDASRV	hESAEKFVEG
A. *terreus* cbs	NLTPFGrNQL	qDlGaQFYRR	YDTLTRhInP	FVRAADSSRV	hESAEKFVEG
A. *niger* var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
A. *niger* T213	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
A. *niger* NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIVP	FIRSSGSSRV	IASGKKFIEG
A. *fumigatus* 13073	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. *fumigatus* 32722	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. *fumigatus* 58128	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. *fumigatus* 26906	DLTAFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. *fumigatus* 32239	DLTPFGEQQM	VNSGIKFYQK	YKALAgSVVP	FIRSSGSDRV	IASGEKFIEG
E. *nidulans*	DLTiFGENQM	VDSGaKFYRR	YKNLARKnTP	FIRASGSDRV	VASAEKFING
T. *thermophilus*	DLTPFGENQM	IQlGIKFYnH	YKSLARNaVP	FVRCSGSDRV	IASGrlFIEG
M. *thermophila*	ELTRtGQQQM	VNSGIKFYRR	YRALARKsIP	FVRTAGqDRV	VhSAENFTQG
Consensus	DLTPFGENQM	VNSGIKFYRR	YKALARK-VP	FVRASGSDRV	IASAEKFIEG
Consensus phytase	DLTPFGENQM	VNSGIKFYRR	YKALARKIVP	FIRASGSDRV	IASAEKFIEG

Fig. 5A

```
                       151                                                  200
A. terreus 9A-1        FQTARqDDHh ANpHQPSPrV DVaIPEGSAY NNTLEHSlCT AFES...STV
A. terreus cbs         FQNARqGDPh ANpHQPSPrV DVVIPEGTAY NNTLEHSICT AFEA...STV
A. niger var. awamori  FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. niger T213          FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. niger NRRL3135      FQSTKLkDPr AqpgQSSPkI DVVISEASSs NNTLDPGTCT VFED...SEL
A. fumigatus 13073     FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 32722     FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 58128     FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 26906     FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT kFEA...SQL
A. fumigatus 32239     FQqANVADPG A.TNRAAPVI SVIIPESETY NNTLDHSVCT NFEA...SEL
E. nidulans            FRKAQLhDHG S..gQATPVV NVIIPEiDGF NNTLDHSTCV SFEN...DEr
T. thermophilus        FQSAKVlDPh SDkHDAPPTI NVIIeEGPSY NNTLDtGSCP VFED...SSg
M. thermophila         FHSAlLADRG STvRPTlPyd mVVIPETAGa NNTLHNDlCT AFEEgpySTI

Consensus              FQSAKLADPG S-PHQASPVI NVIIPEGSGY NNTLDHGTCT AFED---SEL
Consensus phytase      FQSAKLADPG SQPHQASPVI DVIIPEGSGY NNTLDHGTCT AFED...SEL
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                       201                                                  250
A. terreus 9A-1        GDDAvANFTA VFAPAIaQRL EADLPGVqLS TDDVVnLMAM CPFETVSlTD
A. terreus cbs         GDAAADNFTA VFAPAIakRL EADLPGVqLS ADDVVnLMAM CPFETVSlTD
A. niger var. awamori  ADTVEANFTA TFAPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. niger T213          ADTVEANFTA TFAPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. niger NRRL3135      ADTVEANFTA TFVPSIRQRL ENDLSGVTLT DTEVTyLMDM CSFDTIStST
A. fumigatus 13073     GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 32722     GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 58128     GDEVAANFTA lFAPDIRARa EkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 26906     GDEVAANFTA lFAPDIRARa KkHLPGVTLT DEDVVsLMDM CSFDTVARTS
A. fumigatus 32239     GDEVEANFTA lFAPAIRARI EkHLPGVqLT DDDVVsLMDM CSFDTVARTA
E. nidulans            ADEiEANFTA IMGPPIRkRL ENDLPGIKLT NENVIyLMDM CSFDTMARTA
T. thermophilus        GHDAQEKFAk qFAPAIlEKI KDHLPGVDLA vSDVpyLMDL CPFETLARNh
M. thermophila         GDDAQDTYlS TFAGPItARV NANLPGANLT DADTVaLMDL CPFETVAsSS

Consensus              GDDAEANFTA TFAPAIRARL EADLPGVTLT DEDVV-LMDM CPFETVARTS
Consensus phytase      GDDVEANFTA LFAPAIRARL EADLPGVTLT DEDVVYLMDM CPFETVARTS
```

```
                       251                                                  300
A. terreus 9A-1        .......... ...DAhTLSPFC DLFTAtEWtq YNYLlSLDKY YGYGGGNPLG
A. terreus cbs         .......... ...DAhTLSPFC DLFTAaEWtq YNYLlSLDKY YGYGGGNPLG
A. niger var. awamori  .......... ...vDTKLSPFC DLFTHdEWih YDYLQSLkKY YGHGAGNPLG
A. niger T213          .......... ...vDTKLSPFC DLFTHdEWih YDYLRSLkKY YGHGAGNPLG
A. niger NRRL3135      .......... ...vDTKLSPFC DLFTHdEWin YDYLQSLkKY YGHGAGNPLG
A. fumigatus 13073     .......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32722     .......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 58128     .......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 26906     .......... ...DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
A. fumigatus 32239     .......... ...DASELSPFC AIFTHnEWkk YDYLQSLGKY YGYGAGNPLG
E. nidulans            .......... ...HGTELSPFC AIFTEkEWlq YDYLQSLSKY YGYGAGSPLG
T. thermophilus        .......... ...TDT.LSPFC ALsTQeEWqa YDYYQSLGKY YGnGGGNPLG
M. thermophila         sdpatadagg gNGrpLSPFC rLFSEsEWra YDYLQSVGKW YGYGPGNPLG

Consensus              ---------- -DATELSPFC ALFTE-EW-- YDYLQSLGKY YGYGAGNPLG
Consensus phytase      .......... .DATELSPFC ALFTHDEWRQ YDYLQSLGKY YGYGAGNPLG
```

Fig. 5B

350	301
A. terreus 9A-1	PVQGVGWaNE LMARLTRAPV HDHTCVNNTL DASPATFPLN ATLYADFSHD
A. terreus cbs	PVQGVGWaNE LIARLTRSPV HDHTCVNNTL DANPATFPLN ATLYADFSHD
A. niger var. *awamori*	PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSNPATFPLN STLYADFSHD
A. niger T213	PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSNPATFPLN STLYADFSHD
A. niger NRRL3135	PTQGVGYaNE LIARLTHSPV HDDTSSNHTL DSSPATFPLN STLYADFSHD
A. fumigatus 13073	PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 32722	PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 58128	PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 26906	PAQGIGFtNE LIARLTRSPV QDHTSTNsTL vSNPATFPLN ATMYVDFSHD
A. fumigatus 32239	PAQGIGFtNE LIARLTNSPV QDHTSTNsTL DSDPATFPLN ATIYVDFSHD
E. nidulans	PAQGIGFtNE LIARLTQSPV QDNTSTNHTL DSNPATFPLD rKLYADFSHD
T. thermophilus	PAQGVGFvNE LIARMTHSPV QDYTTVNHTL DSNPATFPLN ATLYADFSHD
M. thermophila	PTQGVGFvNE LLARLAgvPV RDgTSTNRTL DGDPrTFPLG rPLYADFSHD
Consensus	PAQGVGF-NE LIARLTHSPV QDHTSTNHTL DSNPATFPLN ATLYADFSHD
Consensus phytase	PAQGVGFANE LIARLTRSPV QDHTSTNHTL DSNPATFPLN ATLYADFSHD

400	351
A. terreus 9A-1	SNLVSIFWAL GLYNGTAPLS qTSVESVSQT DGYAAAWTVP FAARAYVEMM
A. terreus cbs	SNLVSIFWAL GLYNGTkPLS qTTVEDITrT DGYAAAWTVP FAARAYIEMM
A. niger var. *awamori*	NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. niger T213	NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. niger NRRL3135	NGIISILFAL GLYNGTkPLS TTTVENITQT DGFSSAWTVP FASRlYVEMM
A. fumigatus 13073	NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 32722	NSMVSIFFAL GLYNGTGPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 58128	NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 26906	NSMVSIFFAL GLYNGTEPLS rTSVESaKEl DGYSASWVVP FGARAYFEtM
A. fumigatus 32239	NGMIPIFFAM GLYNGTEPLS qTSeESTKES NGYSASWAVP FGARAYFEtM
E. nidulans	NSMISIFFAM GLYNGTQPLS mDSVESIQEm DGYAASWTVP FGARAYFELM
T. thermophilus	NTMTSIFaAL GLYNGTAkLS TTEIKSIEET DGYSAAWTVP FGGRAYIEMM
M. thermophila	NDMMGVLgAL GaYDGVPPLD KTArrDpEEl GGYAASWAVP FAARiYVEKM
Consensus	NSMISIFFAL GLYNGTAPLS TTSVESIEET DGYAASWTVP FGARAYVEMM
Consensus phytase	NSMISIFFAL GLYNGTAPLS TTSVESIEET DGYSASWTVP FGARAYVEMM

450	401
A. terreus 9A-1	QC........ .....RAEKE PLVRVLVNDR VMPLHGCPTD KLGRCKrDAF
A. terreus cbs	QC........ .....RAEKQ PLVRVLVNDR VMPLHGCAVD NLGRCKrDDF
A. niger var. *awamori*	QC........ .....QAEQE PLVRVLVNDR VVPLHGCPID aLGRCTrDSF
A. niger T213	QC........ .....QAEQE PLVRVLVNDR VVPLHGCPID aLGRCTrDSF
A. niger NRRL3135	QC........ .....QAEQE PLVRVLVNDR VVPLHGCPVD aLGRCTrDSF
A. fumigatus 13073	QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32722	QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 58128	QC........ .....KSEKE SLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 26906	QC........ .....KSEKE PLVRALINDR VVPLHGCDVD KLGRCKLNDF
A. fumigatus 32239	QC........ .....KSEKE PLVRALINDR VVPLHGCAVD KLGRCKLKDF
E. nidulans	QC........ .....E.KKE PLVRVLVNDR VVPLHGCAVD KFGRCTLDDW
T. thermophilus	QC........ .....DDSDE PVVRVLVNDR VVPLHGCEVD SLGRCKrDDF
M. thermophila	RCsggggggg ggegrQEKDE eMVRVLVNDR VMTLkGCGAD ErGMCTLErF
Consensus	QC-------- -----QAEKE PLVRVLVNDR VVPLHGCAVD KLGRCKLDDF
Consensus phytase	QC........ .....QAEKE PLVRVLVNDR VVPLHGCAVD KLGRCKRDDF

471	451
A. terreus 9A-1	VAGLSFAQAG GNWADCF--- -
A. terreus cbs	VEGLSFARAG GNWAECF--- -
A. niger var. *awamori*	VrGLSFARSG GDWAECsA-- -
A. niger T213	VrGLSFARSG GDWAECFA-- -
A. niger NRRL3135	VrGLSFARSG GDWAECFA-- -
A. fumigatus 13073	VKGLSWARSG GNWGECFS-- -
A. fumigatus 32722	VKGLSWARSG GNWGECFS-- -
A. fumigatus 58128	VKGLSWARSG GNWGECFS-- -
A. fumigatus 26906	VKGLSWARSG GNWGECFS-- -
A. fumigatus 32239	VKGLSWARSG GNSEQSFS-- -
E. nidulans	VEGLNFARSG GNWkTCFTl- -

Fig. 5C

T. thermophilus	VrGLSFARqG GNWEGCYAas e
M. thermophila	IESMAFARGN GKWDlCFA-- -
Consensus	VEGLSFARSG GNWAECFA-- -
Consensus phytase	VEGLSFARSG GNWAECFA.. .

Fig. 5D

```
                                                                              50
                         1
P. involutus (phyA1)     SvP.KnTAPt FPIPeseQrn WSPYSPYFPL AeYkAPPAGC QInQVNIIQR
P. involutus (phyA2)     SvP.RniAPK FSIPeseQrn WSPYSPYFPL AeYkAPPAGC EInQVNIIQR
T. pubescens             hiPlRdTSAc LdVTrDvQqs WSmYSPYFPa AtYvAPPASC QInQVHIIQR
A. pediades              GgvvQaTfvQ pfFPpQiQds WAAYTPYYPV qaYtPPPkDC KItQVNIIQR
P. lycii                 StQfsfvAAQ LPIPaQntsn WGPYdPFFPV EpYaAPPEGC tVtQVNLIQR

Basidio                  S-P-R-TAAQ LPIP-Q-Q-- WSPYSPYFPV A-Y-APPAGC QI-QVNIIQR

                                                                             100
                         51
P. involutus (phyA1)     HGARFPTSGA TTRIKAGLTK LQGvqnfTDA KFNFIkSfkY dLGnsDLVPF
P. involutus (phyA2)     HGARFPTSGA ATRIKAGLSK LQSvqnfTDP KFDFIkSfTY dLGtsDLVPF
T. pubescens             HGARFPTSGA AkRIQTAVAK LKAAsnyTDP lLAFVtNyTY sLGqDsLVeL
A. pediades              HGARFPTSGA GTRIQAAVkK LQSAktyTDP RLDFLtNyTY tLGhDDLVPF
P. lycii                 HGARWPTSGA rSRqvAAVAK IQmArpfTDP KYEFLnDfvY kFGvADLLPF

Basidio                  HGARFPTSGA ATRIQAAVAK LQSA---TDP KLDFL-N-TY -LG-DDLVPF

                                                                             150
                         101
P. involutus (phyA1)     GAaQSfDAGQ EAFARYSkLV SkNNLPFIRA dGSDRVVDSA TNWTAGFAsA
P. involutus (phyA2)     GAaQSfDAGl EvFARYSkLV SsDNLPFIRS dGSDRVVDTA TNWTAGFAsA
T. pubescens             GAtQSSEAGQ EAFTRYSsLV SaDELPFVRA SGSDRVVATA nNWTAGFAlA
A. pediades              GAlQSSQAGE ETFqRYSfLV SkENLPFVRA SSSNRVVDSA TNWTEGFSaA
P. lycii                 GAnQShQTGt DmYTRYStLf egGDVPFVRA AGdQRVVDSS TNWTAGFGdA

Basidio                  GA-QSSQAGQ EAFTRYS-LV S-DNLPFVRA SGSDRVVDSA TNWTAGFA-A

                                                                             200
                         151
P. involutus (phyA1)     ShNTvqPkLn LILPQtGNDT LEDNMCPaAG DSDPQvNaWL AVafPSITAR
P. involutus (phyA2)     SrNAiqPkLd LILPQtGNDT LEDNMCPaAG ESDPQvDaWL AsafPSVTAQ
T. pubescens             SsNSitPvLs VIISEaGNDT LDDNMCPaAG DSDPQvNqWL AqFAPPMTAR
A. pediades              ShHvlnPiLf VILSEslNDT LDDaMCPnAG sSDPQtGiWt SIYGTPIAnR
P. lycii                 SgETvlPtLq VVLqEeGNcT LcNNMCPnEv DGDest.tWL GVFAPnITAR

Basidio                  S-NT--P-L- VILSE-GNDT LDDNMCP-AG DSDPQ-N-WL AVFAPPITAR

                                                                             250
                         201
P. involutus (phyA1)     LNAAAPSvNL TDtDAfNLvs LCAFlTVSkE kkSdFCtLFE giPGsFeAFa
P. involutus (phyA2)     LNAAAPGANL TDaDAfNLvs LCPFmTVSkE qkSdFCtLFE giPGsFeAFa
T. pubescens             LNAGAPGANL TDtDTyNLlt LCPFETVAtE rrSeFCDIYE elQAE.dAFa
A. pediades              LNqqAPGANI TAaDvsNLip LCAFETIvkE tpSpFCNLF. .tPEEFaqFe
P. lycii                 LNAAAPSANL SDsDAltLmd MCPFDTLSsG naSpFCDLF. .tAEEYvSYe

Basidio                  LNAAAPGANL TD-DA-NL-- LCPFETVS-E --S-FCDLFE --PEEF-AF-

                                                                             300
                         251
P. involutus (phyA1)     YgGDLDKFYG TGYGQeLGPV QGVGYVNELI ARLTnsAVRD NTQTNRTLDA
P. involutus (phyA2)     YaGDLDKFYG TGYGQALGPV QGVGYINELL ARLTnsAVnD NTQTNRTLDA
T. pubescens             YnADLDKFYG TGYGQPLGPV QGVGYINELI ARLTaQnVsD HTQTNsTLDS
A. pediades              YfGDLDKFYG TGYGQPLGPV QGVGYINELL ARLTemPVRD NTQTNRTLDS
P. lycii                 YyyDLDKYYG TGpGNALGPV QGVGYVNELL ARLTgQAVRD ETQTNRTLDS

Basidio                  Y-GDLDKFYG TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRTLDS

                                                                             350
                         301
```

Fig. 6A

```
P. involutus (phyA1) SPvTFPLNKT FYADFSHDNl MVAVFSAMGL FrQPAPLsTS vPNPwRTWrT
P. involutus (phyA2) APdTFPLNKT MYADFSHDNl MVAVFSAMGL FrQSAPLsTS tPDPNRTWLT
T. pubescens         SPeTFPLNRT LYADFSHDNQ MVAIFSAMGL FNQSAPLDPT tPDPaRTFLv
A. pediades          SPlTFPLDRS IYADLSHDNQ MIAIFSAMGL FNQSSPLDPS fPNPKRTWVT
P. lycii             dPaTFPLNRT FYADFSHDNt MVPIFAALGL FNaTA.LDPl kPDeNRlWVd

Basidio              SP-TFPLNRT FYADFSHDNQ MVAIFSAMGL FNQSAPLDPS -PDPNRTWVT


                     351                                                400
P. involutus (phyA1) SsLVPFSGRM VVERLsC..f GT........ .......tkV RVLVQDqVQP
P. involutus (phyA2) SsVVPFSARM aVERLsC..a GT........ .......tkV RVLVQDqVQP
T. pubescens         kKIVPFSARM VVERLdC..g GA........ .......qsV RLLVNDAVQP
A. pediades          SRLtPFSARM VtERLlCqrd GTgsggpsri mrngnvqtfV RILVNDALQP
P. lycii             SKLVPFSGHM tVEKLaC... .......... ....sgkeaV RVLVNDAVQP

Basidio              SKLVPFSARM VVERL-C--- GT-------- ---------V RVLVNDAVQP


                     401                                       441
P. involutus (phyA1) LEFCGGDrNG lCTLAkFVES QtFARsDGaG DFEKCFATSa -
P. involutus (phyA2) LEFCGGDqDG lCALDkFVES QaYARsGGaG DFEKCLATTv -
T. pubescens         LAFCGADtsG vCTLDAFVES QaYARNDGEG DFEKCFAT-- -
A. pediades          LKFCGGDmDS lCTLEAFVES QkYAREDGQG DFEKCFD--- -
P. lycii             LEFCGG.vDG vCeLsAFVES QtYARENGQG DFAKCgfvPs e

Basidio              LEFCGGD-DG -CTLDAFVES Q-YAREDGQG DFEKCFATP- -
```

Fig. 6B

	1				50
A. terreus 9a1	KhsdCNSVDh	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPeDCHITFV
A. terreus cbs	NhsdCtSVDr	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPdDCHITFV
A. niger var. *awamori*	NqsTCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESAISPD	VPaGCRVTFa
A. niger NRRL3135	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPE	VPaGCRVTFa
A. fumigatus 13073	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 32722	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 58128	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 26906	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
A. fumigatus 32239	GSkACDTVEl	GYQCsPGtSH	LWGQYSPFFS	LEDElSVSSD	LPkDCRVTFV
E. nidulans	QNHSCNTaDG	GYQCfPNVSH	VWGQYSPYFS	IEQESAISeD	VPhGCeVTFV
T. thermophilus	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPqNCKITFV
T. lanuginosa	----------	----nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPkGCRVeFV
M. thermophila	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPdDCeVTFa
Basidio	xSxPxrxtAA	qLPipxQxqx	xWSPYSPYFP	VAxyxA....	pPaGCQIxqV
Consensus	NSHSCDTVDG	GYQC-PEISH	LWGQYSPFFS	LADESAISPD	VP-GCRVTFV
Fcp10	NSHSCDTVDG	GYQCFPEISH	LWGQYSPFFS	LADESAISPD	VPKGCRVTFV

	51				100
A. terreus 9a1	QVLARHGARs	PThSKTKaYA	AtIaAIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. terreus cbs	QVLARHGARs	PTdSKTKaYA	AtIaAIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. niger var. *awamori*	QVLSRHGARY	PTeSKGKKYS	ALIeEIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. niger NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIeEIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. fumigatus 13073	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32722	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 58128	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 26906	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. fumigatus 32239	QVLSRHGARY	PTASKSKKYk	kLVtAIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. nidulans	QVLSRHGARY	PTeSKSKaYS	GLIeAIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. thermophilus	QLLSRHGARY	PTSSKTElYS	qLIsrIQKtA	TaYKGyYAFL	KdYrYqLGAN
T. lanuginosa	QVLSRHGARY	PTAhKSEvYA	ELLqrIQDtA	TeFKGDFAFL	RdYaYhLGAD
M. thermophila	QVLSRHGARa	PTlkRAasYv	DLIdrIHhGA	isYgPgYEFL	RTYDYTLGAD
Basidio	NIIqRHGARF	PTSGaAtRiq	AaVakLQsax	xxtDPKLDFL	xnxtYxLGxD
Consensus	QVLSRHGARY	PTSSKSKKYS	ALI-AIQKNA	T-FKGKYAFL	KTYNYTLGAD
Fcp10	QVLSRHGARY	PTSSKSKKYS	ALIEAIQKNA	TAFKGKYAFL	KTYNYTLGAD

	101				150
A. terreus 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRhIn	PFVRATDAsR	VhESAEKFVE
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRhIn	PFVRAADSsR	VhESAEKFVE
A. niger var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnII	PFIRSSGSsR	VIASGEKFIE
A. niger NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnIV	PFIRSSGSsR	VIASGKKFIE
A. fumigatus 13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
A. fumigatus 32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgsVV	PFIRSSGSDR	VIASGEKFIE
E. nidulans	DLTiFGENQM	VDSGaKFYRR	YKnL.ARknt	PFIRASGSDR	VVASAEKFIN
T. thermophilus	DLTPFGENQM	IQlGIKFYnH	YKSL.ARnaV	PFVRCSGSDR	VIASGrlFIE
T. lanuginosa	NLTRFGEEQM	MESGrQFYHR	YREq.AReIV	PFVRAAGSAR	VIASAEfFnr
M. thermophila	ELTRtGQQQM	VNSGIKFYRR	YRAL.ARksI	PFVRTAGqDR	VVhSAENFtQ
Basidio	DLvPFGAxQs	sQAGqEaFtR	YsxLvSxdnL	PFVRASGSDR	VVDSAtNWtA
Consensus	DLTPFGEQQM	VNSGIKFYRR	YKAL-AR-IV	PFVRASGSDR	VIASAEKFIE

Fig. 7A

```
            Fcp10   DLTPFGEQQM VNSGIKFYRR YKAL.ARKIV PFVRASGSDR VIASAEKFIE

                    151                                                200
A. terreus 9a1            GFQTARqDDh hAnphQPSPr VDVaIPEGsA YNNTLEHSLC TAFEs...St
A. terreus cbs            GFQNARqGDP hAnphQPSPr VDVVIPEGtA YNNTLEHSIC TAFEa...St
A. niger var. awamori     GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. niger NRRL3135         GFQSTKLkDP rAqpgQSSPk IDVVISEAsS sNNTLDpGtC TvFEd...SE
A. fumigatus 13073        GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32722        GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 58128        GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 26906        GFQqAKLADP gAt.nRAAPa ISVIIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32239        GFQqANVADP gAt.nRAAPV ISVIIPESeT YNNTLDHSVC TnFEa...SE
E. nidulans               GFRkAQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC vSFEn...dE
T. thermophilus           GFQSAKVlDP hSdkhDAPPt INVIIeEGpS YNNTLDtGsC PvFEd...Ss
T. lanuginosa             GFQdAKdrDP rSnkdQAePV INVIISEEtG sNNTLDgltC PAaEe...Ap
M. thermophila            GFHSAlLADR gStvrPTlPy dmVVIPETaG aNNTLHNDLC TAFEegPySt
Basidio                   GFaxA..... ..sxntxxPx LxVILSExg. .NDTLDDNMC ......PxAG

        Consensus   GFQSAKLADP -A---QASPV INVIIPEG-G YNNTLDHGLC TAFE--P-SE
            Fcp10   GFQSAKLADP GANPHQASPV INVIIPEGAG YNNTLDHGLC TAFEE...SE

                    201                                                250
A. terreus 9a1            VGDDavANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA MCPFETVSlT
A. terreus cbs            VGDAaADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA MCPFETVSlT
A. niger var. awamori     LADtVEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTISt S
A. niger NRRL3135         LADtVEANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS
A. fumigatus 13073        LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 32722        LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 58128        LGDEVAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 26906        LGDEVAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD MCSFDTVArT
A. fumigatus 32239        LGDEVEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD MCSFDTVArT
E. nidulans               rADEIEANFT AIMGPPIRkR LEndLPGIKL TNENVIyLMD MCSFDTMArT
T. thermophilus           gGHDaQEKFA kqFAPAIlEK IKDhLPGVDL AvsDVpyLMD LCPFETLArn
T. lanuginosa             .DptqpAEFl qVFGPRVlkK ItkhMPGVNL TlEDVplFMD LCPFDTVGsd
M. thermophila            IGDDaQDtYl StFAGPItAR VNAnLPGaNL TDADtVaLMD LCPFETVAsS
Basidio                   dSDpqxnxWl AVFAPPItAR LNAaaPGaNL TDxDaxNLxx LCPFETVS..

        Consensus   LGDDVEANFT AVFAPPIRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVA-T
            Fcp10   LGDDVEANFT AVFAPPIRAR LEAHLPGVNL TDEDVVNLMD MCPFDTVART

                    251                                                300
A. terreus 9a1            dD..Aht... ......LSPF CDLFTa..tE WtQYNYLlSL dKYYGYGGGN
A. terreus cbs            dD..Aht... ......LSPF CDLFTa..aE WtQYNYLlSL dKYYGYGGGN
A. niger var. awamori     Tv..DTK... ......LSPF CDLFTH..dE WiHYDYLQSL kKYYGHGAGN
A. niger NRRL3135         Tv..DTK... ......LSPF CDLFTH..dE WiNYDYLQSL kKYYGHGAGN
A. fumigatus 13073        SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 32722        SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 58128        SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 26906        SD..ASQ... ......LSPF CQLFTH..nE WkKYNYLQSL gKYYGYGAGN
A. fumigatus 32239        AD..ASE... ......LSPF CAIFTH..nE WkKYDYLQSL gKYYGYGAGN
E. nidulans               AH..GTE... ......LSPF CAIFTE..kE WlQYDYLQSL sKYYGYGAGS
T. thermophilus           ht..DT.... ......LSPF CALsTQ..eE WqaYDYYQSL gKYYGnGGGN
T. lanuginosa             PvlfPrQ... ......LSPF CHLFTa..dD WmaYDYYyTL dKYYSHGGGS
M. thermophila            SsdpATadag ggngrpLSPF CrLFSE..sE WraYDYLQSV gKWYGYGPGN
Basidio                   .......... ...xexxSxF CDLFexxpeE FxaFxYxgdL dKFYGtGyGQ

        Consensus   SD--ATQ--- ------LSPF CDLFTH---E W-QYDYLQSL -KYYGYGAGN
            Fcp10   SD..ATQ... ......LSPF CDLFTH..DE WIQYDYLQSL GKYYGYGAGN

                    301                                                350
A. terreus 9a1            PLGPvQGVGW aNELMARLTR A.PVHDHTCv NNTLDASPAT FPLNATLYAD
A. terreus cbs            PLGPvQGVGW aNELIARLTR S.PVHDHTCv NNTLDANPAT FPLNATLYAD
A. niger var. awamori     PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD
A. niger NRRL3135         PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD
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Fig. 7B

A. fumigatus 13073	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 32722	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 58128	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 26906	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
A. fumigatus 32239	PLGPAQGIGF	tNELIARLTN	S.PVQDHTST	NsTLDSDPAT	FPLNATIYvD
E. nidulans	PLGPAQGIGF	tNELIARLTQ	S.PVQDNTST	NHTLDSNPAT	FPLDrkLYAD
T. thermophilus	PLGPAQGVGF	vNELIARMTH	S.PVQDYTTv	NHTLDSNPAT	FPLNATLYAD
T. lanuginosa	AFGPSRGVGF	vNELIARMTg	NlPVKDHTTv	NHTLDdNPET	FPLDAvLYAD
M. thermophila	PLGPTQGVGF	vNELLARLA.	GvPVRDgTST	NRTLDGDPrT	FPLGrPLYAD
Basidio	PLGPvQGVGY	iNELLARLTx	qa.VRDNTqT	NRTLDSSPxT	FPLNrTFYAD
Consensus	PLGPAQGVGF	-NELIARLTH	S-PVQDHTST	NHTLDSNPAT	FPLNATLYAD
Fcp10	PLGPAQGVGF	VNELIARLTH	S.PVQDHTST	NHTLDSNPAT	FPLNATLYAD

	351				400
A. terreus 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqTSVE.	.SvsQTDGYA	AAWTVPFAAR
A. terreus cbs	FSHDSnLVSI	FWALGLYNGT	kPLSqTTVE.	.ditrTDGYA	AAWTVPFAAR
A. niger var. *awamori*	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS	SAWTVPFASR
A. niger NRRL3135	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS	SAWTVPFASR
A. fumigatus 13073	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 32722	FSHDNSMVSI	FFALGLYNGT	gPLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 58128	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 26906	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS	ASWvVPFGAR
A. fumigatus 32239	FSHDNGMIPI	FFAMGLYNGT	ePLSqTSeE.	.StKESNGYS	ASWAVPFGAR
E. nidulans	FSHDNSMISI	FFAMGLYNGT	qPLSmdSVE.	.SiQEmDGYA	ASWTVPFGAR
T. thermophilus	FSHDNTMtSI	FaALGLYNGT	akLSTTeIK.	.SiEETDGYS	AAWTVPFGGR
T. lanuginosa	FSHDNTMtGI	FsAMGLYNGT	kPLSTSkIQP	pTgAAADGYA	ASWTVPFAAR
M. thermophila	FSHDNdMMGV	LgALGaYDGv	pPLdkTA..R	rdpEElGGYA	ASWAVPFAAR
Basidio	FSHDNqMVAI	FsAMGLFNqS	aPLdPSxpDP	nrt.....Wv	TSklVPFSAR
Consensus	FSHDNTMVSI	FFALGLYNGT	-PLSTTSVEP	-S-EETDGYA	ASWTVPFAAR
Fcp10	FSHDNTMVSI	FFALGLYNGT	KPLSTTSVE.	.SIEETDGYA	ASWTVPFAAR

	401				450
A. terreus 9a1	AYVEMMQC..	ra........	.....EKEPL	VRVLVNDRVM	PLHGCPtDKL
A. terreus cbs	AYIEMMQC..	ra........	.....EKQPL	VRVLVNDRVM	PLHGCAVDNL
A. niger var. *awamori*	lYVEMMQC..	Qa........	.....EQEPL	VRVLVNDRVV	PLHGCPIDaL
A. niger NRRL3135	lYVEMMQC..	Qa........	.....EQEPL	VRVLVNDRVV	PLHGCPVDaL
A. fumigatus 13073	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 32722	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 58128	AYfEtMQC..	Ks........	.....EKESL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 26906	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus 32239	AYfEtMQC..	Ks........	.....EKEPL	VRaLINDRVV	PLHGCAVDKL
E. nidulans	AYfELMQC..	E.........	.....KKEPL	VRVLVNDRVV	PLHGCAVDKF
T. thermophilus	AYIEMMQC..	Dd........	.....sDEPV	VRVLVNDRVV	PLHGCEVDsL
T. lanuginosa	AYVELLRC..	Etetsseeee	EG...EDEPF	VRVLVNDRVV	PLHGCrVDRW
M. thermophila	iYVEkMRC..	sggggggggg	EGrqeKDEeM	VRVLVNDRVM	TLkGCGaDEr
Basidio	mvVErLxCxx	xgtxxxxxxx	xxxxxxxxxx	VRVLVNDaVq	PLEfCGgDxd
Consensus	AYVEMMQC--	E---------	EG---EKEPL	VRVLVNDRVV	PLHGCGVDKL
Fcp10	AYVEMMQC..	EA........	.....EKEPL	VRVLVNDRVV	PLHGCGVDKL

Fig. 7C

	451		482
A. terreus 9a1	GRCKrDAFVA	GLSFAQAG..	GNWADCF--- --
A. terreus cbs	GRCKrDDFVE	GLSFARAG..	GNWAECF--- --
A. niger var. *awamori*	GRCtrDsFVr	GLSFARSG..	GDWAECsA-- --
A. niger NRRL3135	GRCtrDsFVr	GLSFARSG..	GDWAECFA-- --
A. fumigatus 13073	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 32722	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 58128	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 26906	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 32239	GRCKlKDFVK	GLSWARSG..	GNSEQSFS-- --
E. nidulans	GRCtlDDWVE	GLNFARSG..	GNWKtCFTl- --
T. thermophilus	GRCKrDDFVr	GLSFARqG..	GNWEGCYAas e-
T. lanuginosa	GRCRrDEWIK	GLTFARqG..	GHWDrCF--- --
M. thermophila	GmCtlErFIE	SMAFARGN..	GKWDlCFA-- --
Basidio	GxCtlDAFVE	SqxYAReDgq	GDFEKCFAtp xx
Consensus	GRCK-DDFVE	GLSFARSG--	GNWEECFA-- --
Fcp10	**GRCKRDDFVE**	**GLSFARSG..**	**GNWEECFA.. ..**

Fig. 7D

15/32

	1				50
P. *involutus* (*phyA1*)	----------	-FPipeseqR	nWSPYSPYFP	LAEykA....	pPaGCQInqV
P. *involutus* (*phyA2*)	----------	-FsipeseqR	nWSPYSPYFP	LAEykA....	pPaGCeInqV
T. *pubescens*	----------	-LDvtRDVqQ	sWSmYSPYFP	aAtyvA....	pPaSCQInqV
A. *pediades*	----------	-pffpPQIqD	sWAaYTPYYP	VqAyTP....	pPKDCKITqV
P. *lycii*	----------	-LPipAQnTs	nWGPYdPFFP	VEpyAA....	pPEGCtVTqV
A. *terreus* 9a1	KhsdCNSVDh	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPEDCHITFV
A. *terreus* cbs	NhsdCtSVDr	GYQCfPELSH	kWGlYAPYFS	LqDESPFPlD	VPDDCHITFV
A. *niger* var. *awamori*	NqsTCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESAISPD	VPaGCRVTFa
A. *niger* T213	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPD	VPaGCRVTFa
A. *niger* NRRL3135	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPE	VPaGCRVTFa
A. *fumigatus* ATCC13073	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. *fumigatus* ATCC32722	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. *fumigatus* ATCC58128	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. *fumigatus* ATCC26906	GSkSCDTVDl	GYQCsPAtSH	LWGQYSPFFS	LEDElSVSSK	LPKDCRITLV
A. *fumigatus* ATCC32239	GSkACDTVEl	GYQCsPGtSH	LWGQYSPFFS	LEDElSVSSD	LPKDCRVTFV
E. *nidulans*	QNHSCNTaDg	GYQCfPNVSH	VWGQYSPYFS	IEQESAISeD	VPhGCeVTFV
T. *thermophilus*	DSHSCNTVEg	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCKITFV
T. *lanuginosa*	----------	----nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPKGCRVeFV
M. *thermophila*	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPDDCeVTFa
Consensus Seq. 11	**NSHSCDTVD-**	**GYQC-PEISH**	**LWGQYSPFFS**	**LADESAISPD**	**VPKGCRVTFV**

	51				100
P. *involutus* (*phyA1*)	NIIqRHGARF	PTSGaTtRik	AgLtKLQgvq	nftDAKFnFI	KSFKYdLGns
P. *involutus* (*phyA2*)	NIIqRHGARF	PTSGaAtRik	AgLsKLQsvq	nftDPKFDFI	KSFtYdLGTs
T. *pubescens*	HIIqRHGARF	PTSGaAKRiq	TaVAKLKaaS	nytDPlLAFV	tnYtYSLGqD
A. *pediades*	NIIqRHGARF	PTSGaGtRiq	AaVKKLQsak	TytDPRLDFL	tnYtYTLGhD
P. *lycii*	NLIqRHGARW	PTSGarsRqv	AaVAKIQmar	PftDPKYEFL	NdFvYkFGvA
A. *terreus* 9a1	QVLARHGARs	PThSKTKaYA	AtIAaIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. *terreus* cbs	QVLARHGARs	PTdSKTKaYA	AtIAaIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. *niger* var. *awamori*	QVLSRHGARY	PTeSKGKKYS	ALIEeIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. *niger* T213	QVLSRHGARY	PTeSKGKKYS	ALIEeIQQNv	TtFDGKYAFL	KTYNYSLGAD
A. *niger* NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIEeIQQNA	TtFDGKYAFL	KTYNYSLGAD
A. *fumigatus* ATCC13073	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* ATCC32722	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* ATCC58128	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* ATCC26906	QVLSRHGARY	PTSSKSKKYk	kLVtaIQaNA	TdFKGKFAFL	KTYNYTLGAD
A. *fumigatus* ATCC32239	QVLSRHGARY	PTASKSKKYk	kLVtaIQKNA	TeFKGKFAFL	ETYNYTLGAD
E. *nidulans*	QVLSRHGARY	PTeSKSKaYS	GLIEaIQKNA	TsFwGQYAFL	ESYNYTLGAD
T. *thermophilus*	QLLSRHGARY	PTSSKTElYS	qLIsRIQKtA	TaYKGyYAFL	KdYrYqLGAN
T. *lanuginosa*	QVLSRHGARY	PTAhKSEvYA	ELLQRIQDtA	TeFKGDFAFL	RdYaYhLGAD
M. *thermophila*	QVLSRHGARa	PTlkRAasYv	DLIDRIHhGA	isYgPgYEFL	RTYDYTLGAD
Consensus Seq. 11	**QVLSRHGARY**	**PTSSKSKKYS**	**ALIERIQKNA**	**T-FKGKYAFL**	**KTYNYTLGAD**

101 150					
P. *involutus* (*phyA1*)	DLvPFGAaQs	fDAGqEaFaR	YskLvSKNnL	PFIRAdGSDR	VVDSAtNWtA
P. *involutus* (*phyA2*)	DLvPFGAaQs	fDAGLEvFaR	YskLvSsDnL	PFIRSdGSDR	VVDTAtNWtA
T. *pubescens*	sLveLGAtQs	sEAGqEaFtR	YsSLvSaDeL	PFVRASGSDR	VVATANNWtA
A. *pediades*	DLvPFGAlQs	sQAGeEtFQR	YsfLvSKEnL	PFVRASSSNR	VVDSAtNWtE
P. *lycii*	DLlPFGANQs	hQTGtDMYtR	YsTLfEgGdV	PFVRAAGdQR	VVDSStNWtA
A. *terreus* 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRHIn	PFVRATDAsR	VhESAEKFVE
A. *terreus* cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRHIn	PFVRAADSsR	VhESAEKFVE
A. *niger* var. *awamori*	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
A. *niger* T213	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
A. *niger* NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRNIV	PFIRSSGSsR	VIASGKKFIE
A. *fumigatus* ATCC13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. *fumigatus* ATCC32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
A. *fumigatus* ATCC58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE

Fig. 8A

A. fumigatus ATCC26906	DLTAFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE
A. fumigatus ATCC32239	DLTPFGEQQM VNSGIKFYQK YKAL.AgSVV PFIRSSGSDR VIASGEKFIE
E. nidulans	DLTiFGENQM VDSGaKFYRR YKnL.ARKnt PFIRASGSDR VVASAEKFIN
T. thermophilus	DLTPFGENQM IQlGIKFYnH YKSL.ARNaV PFVRCSGSDR VIASGrlFIE
T. lanuginosa	NLTRFGEEQM MESGrQFYHR YREq.AREIV PFVRAAGSAR VIASAEfFnr
M. thermophila	ELTRtGQQQM VNSGIKFYRR YRAL.ARKsI PFVRTAGqDR VVhSAENFtQ
Consensus Seq. 11	**DLTPFGENQM VNSGIKFYRR YKAL-ARNIV PFVRASGSDR VIASAEKFIE**

	151 200
P. involutus (phyA1)	GFaSA..... ..shNtvqPk LNLILPQ..T gNDTLEDNMC PAaGD.....
P. involutus (phyA2)	GFaSA..... ..srNaiqPk LDLILPQ..T gNDTLEDNMC PAaGE.....
T. pubescens	GFalA..... ..ssNsiTPV LSVIISE..A gNDTLDDNMC PAaGD.....
A. pediades	GFsAA..... ..shHvlNPI LfVILSE..S LNDTLDDAMC PnaGs.....
P. lycii	GFgdA..... ..sgEtvlPt LQVVLQE..E gNcTLcNNMC PnevD.....
A. terreus 9a1	GFQTARqDDh hAnpHQPSPr VDVaIPEGSA YNNTLEHSLC TAFEs...ST
A. terreus cbs	GFQNARqGDP hAnpHQPSPr VDVVIPEGTA YNNTLEHSIC TAFEA...ST
A. niger var. *awamori*	GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC TvFED...Se
A. niger T213	GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC TvFED...Se
A. niger NRRL3135	GFQSTKLkDP rAqpgQSSPk IDVVISEASS sNNTLDpGtC TvFED...Se
A. fumigatus ATCC13073	GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC TkFEA...Sq
A. fumigatus ATCC32722	GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC TkFEA...Sq
A. fumigatus ATCC58128	GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC TkFEA...Sq
A. fumigatus ATCC26906	GFQqAKLADP gAt.NRAAPa ISVIIPESeT FNNTLDHGVC TkFEA...Sq
A. fumigatus ATCC32239	GFQqANVADP gAt.NRAAPV ISVIIPESeT YNNTLDHSVC TnFEA...Se
E. nidulans	GFRkAQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC vSFEN...de
T. thermophilus	GFQSAKVlDP hSdkHDAPPt INVIIeEGPS YNNTLDtGsC PvFED...SS
T. lanuginosa	GFQdAKdrDP rSnkDQAePV INVIISEETG sNNTLDgltC PAaEE...AP
M. thermophila	GFHSAlLADR gStvRPTlPy dmVVIPETAG aNNTLHNDLC TAFEEgpyST
Consensus Seq. 11	**GFQSAKLADP -A--HQASPV INVIIPEGSG YNNTLDHGLC TAFED---ST**

	201 250
P. involutus (phyA1)	.SDpqvnaWl AVafPSItAR LNAaaPSVNL TDtDafNLVs LCAFlTVSK.
P. involutus (phyA2)	.SDpqvDaWl AsafPSVtAQ LNAaaPGaNL TDADafNLVs LCPFmTVSK.
T. pubescens	.SDpqvnQWl AqFAPPMtAR LNAgaPGaNL TDtDtyNLLt LCPFETVAt.
A. pediades	.SDpqtGiWT SIYGTPIanR LNqqaPGaNI TAADVsNLIp LCAFETIvK.
P. lycii	.GDESt.tWl GVFAPnItAR LNAaaPSaNL SDsDaLtLMD MCPFDTLSs.
A. terreus 9a1	VGDDAvANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA MCPFETVSlT
A. terreus cbs	VGDAAADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA MCPFETVSlT
A. niger var. *awamori*	LADtvEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS
A. niger T213	LADtvEANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS
A. niger NRRL3135	LADtvEANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIStS
A. fumigatus ATCC13073	LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART
A. fumigatus ATCC32722	LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART
A. fumigatus ATCC58128	LGDEvAANFT ALFAPdIRAR aEkhLPGVtL TDEDVVSLMD MCSFDTVART
A. fumigatus ATCC26906	LGDEvAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD MCSFDTVART
A. fumigatus ATCC32239	LGDEvEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD MCSFDTVART
E. nidulans	rADEiEANFT AIMGPPIRkR LEndLPGIKL TNENVIyLMD MCSFDTMART
T. thermophilus	gGHDAQEKFA kqFAPAIlEK IKDhLPGVDL AvsDVpyLMD LCPFETLARn
T. lanuginosa	.DptqpAEFl qVFGPRVlkK ItkhMPGVNL TlEDVplFMD LCPFDTVGsd
M. thermophila	IGDDAQDtYl StFAGPItAR VNAnLPGaNL TDADtVaLMD LCPFETVAsS
Consensus Seq. 11	**LGDDAEANFT AVFAPPIRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVART**

	251 300
P. involutus (phyA1)	....ekkSdF CtLFegiPGs FeaFAYggdL dKFYGtGyGQ
P. involutus (phyA2)	....eqkSdF CtLFegiPGs FeaFAYagdL dKFYGtGyGQ
T. pubescens	....errSeF CDIYeelqAE .daFAYnadL dKFYGtGyGQ
A. pediades	....etpSPF CNLF..TPEE FaQFEYFgdL dKFYGtGyGQ
P. lycii	....gnaSPF CDLF..TAEE YvsYEYYydL dKYYGtGPGN
A. terreus 9a1	dD..Aht... ......LSPF CDLF..TAtE WtQYNYLlSL dKYYGYGGGN
A. terreus cbs	dD..Aht... ......LSPF CDLF..TAAE WtQYNYLlSL dKYYGYGGGN
A. niger var. *awamori*	Tv..DTK... ......LSPF CDLF..ThDE WiHYDYLQSL kKYYGHGAGN

Fig. 8B

```
A. niger T213              Tv..DTK... ......LSPF CDLF..ThDE WiHYDYLRSL kKYYGHGAGN
A. niger NRRL3135          Tv..DTK... ......LSPF CDLF..ThDE WiNYDYLQSL kKYYGHGAGN
A. fumigatus ATCC13073     SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL gKYYGYGAGN
A. fumigatus ATCC32722     SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL gKYYGYGAGN
A. fumigatus ATCC58128     SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL gKYYGYGAGN
A. fumigatus ATCC26906     SD..ASQ... ......LSPF CQLF..ThNE WkKYNYLQSL gKYYGYGAGN
A. fumigatus ATCC32239     AD..ASE... ......LSPF CAIF..ThNE WkKYDYLQSL gKYYGYGAGN
E. nidulans                AH..GTE... ......LSPF CAIF..TEKE WlQYDYLQSL sKYYGYGAGS
T. thermophilus            ht..DT.... ......LSPF CALs..TqEE WqaYDYYQSL gKYYGnGGGN
T. lanuginosa              PvlfPrQ... ......LSPF CHLF..TADD WmaYDYYyTL dKYYSHGGGS
M. thermophila             SsdpATadag ggngrpLSPF CrLF..SEsE WraYDYLQSV gKWYGYGPGN

Consensus Seq. 11          SD--ATQ--- ------LSPF CDLF--TADE W-QYDYLQSL -KYYGYGAGN

                           301                                               350
P. involutus (phyA1)       eLGPvQGVGY vNELIARLTN S.AVRDNTqT NRTLDASPvT FPLNkTFYAD
P. involutus (phyA2)       ALGPvQGVGY iNELLARLTN S.AVNDNTqT NRTLDAaPDT FPLNkTMYAD
T. pubescens               PLGPvQGVGY iNELIARLTa q.nVsDHTqT NsTLDSSPET FPLNrTLYAD
A. pediades                PLGPvQGVGY iNELLARLTE m.PVRDNTqT NRTLDSSPlT FPLDrSIYAD
P. lycii                   ALGPvQGVGY vNELLARLTg q.AVRDETqT NRTLDSDPAT FPLNrTFYAD
A. terreus 9a1             PLGPvQGVGW aNELMARLTR A.PVHDHTCv NNTLDASPAT FPLNATLYAD
A. terreus cbs             PLGPvQGVGW aNELIARLTR S.PVHDHTCv NNTLDANPAT FPLNATLYAD
A. niger var. awamori      PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD
A. niger T213              PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD
A. niger NRRL3135          PLGPTQGVGY aNELIARLTH S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD
A. fumigatus ATCC13073     PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD
A. fumigatus ATCC32722     PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD
A. fumigatus ATCC58128     PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD
A. fumigatus ATCC26906     PLGPAQGIGF tNELIARLTR S.PVQDHTST NsTLvSNPAT FPLNATMYvD
A. fumigatus ATCC32239     PLGPAQGIGF tNELIARLTN S.PVQDHTST NsTLDSDPAT FPLNATIYvD
E. nidulans                PLGPAQGIGF tNELIARLTQ S.PVQDNTST NHTLDSNPAT FPLDrkLYAD
T. thermophilus            PLGPAQGVGF vNELIARMTH S.PVQDYTTv NHTLDSNPAT FPLNATLYAD
T. lanuginosa              AFGPSRGVGF vNELIARMTg NlPVKDHTTv NHTLDdNPET FPLDAvLYAD
M. thermophila             PLGPTQGVGF vNELLARLA. GvPVRDgTST NRTLDGDPrT FPLGrPLYAD

Consensus Seq. 11          PLGPAQGVGF -NELIARLTH S-PVQDHTST NHTLDSNPAT FPLNATLYAD

                           351                                               400
P. involutus (phyA1)       FSHDNlMVAV FsAMGLFrqP aPLSTSvpNP wrt.....Wr TSSlVPFSGR
P. involutus (phyA2)       FSHDNlMVAV FsAMGLFrqS aPLSTSTpDP nrt.....Wl TSSvVPFSAR
T. pubescens               FSHDNqMVAI FsAMGLFNqS aPLdPTTpDP art.....Fl vkkiVPFSAR
A. pediades                LSHDNqMIAI FsAMGLFNqS sPLdPSfpNP krt.....Wv TSRltPFSAR
P. lycii                   FSHDNTMVPI FaALGLFNAT a.LdPlkpDe nrl.....Wv DSklVPFSGH
A. terreus 9a1             FSHDSnLVSI FWALGLYNGT aPLSqTSVES Vs..QTDGYA AAWTVPFAAR
A. terreus cbs             FSHDSnLVSI FWALGLYNGT KPLSqTTVEd It..rTDGYA AAWTVPFAAR
A. niger var. awamori      FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR
A. niger T213              FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR
A. niger NRRL3135          FSHDNGIISI LFALGLYNGT KPLSTTTVEN It..QTDGFS SAWTVPFASR
A. fumigatus ATCC13073     FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR
A. fumigatus ATCC32722     FSHDNSMVSI FFALGLYNGT gPLSrTSVES ak..ElDGYS ASWvVPFGAR
A. fumigatus ATCC58128     FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR
A. fumigatus ATCC26906     FSHDNSMVSI FFALGLYNGT EPLSrTSVES ak..ElDGYS ASWvVPFGAR
A. fumigatus ATCC32239     FSHDNGMIPI FFAMGLYNGT EPLSqTSeES tk..ESNGYS ASWAVPFGAR
E. nidulans                FSHDNSMISI FFAMGLYNGT QPLSmdSVES Iq..EmDGYA ASWTVPFGAR
T. thermophilus            FSHDNTMtSI FaALGLYNGT akLSTTeIKS Ie..ETDGYS AAWTVPFGGR
T. lanuginosa              FSHDNTMtGI FsAMGLYNGT KPLSTSkIQP ptgaAADGYA ASWTVPFAAR
M. thermophila             FSHDNdMMGV LgALGaYDGv pPLdkTArrd ..peElGGYA ASWAVPFAAR

Consensus Seq. 11          FSHDNTMVSI FFALGLYNGT KPLSTTSVES I---ETDGYA ASWTVPFAAR

                           401                                               450
P. involutus (phyA1)       mvVErLsC.. fGt....... ........Tk VRVLVQDQVq PLEfCGgDRn
P. involutus (phyA2)       maVErLsC.. AGt....... ........Tk VRVLVQDQVq PLEfCGgDQd
T. pubescens               mvVErLDC.. GGa....... ........Qs VRLLVNDaVq PLafCGaDts
```

Fig. 8C

A. pediades	mvtErLlCQr	DGtGsGGpsr	imrNgnvQTF	VRILVNDaLq	PLkfCGgDmd
P. lycii	mtVEkLaC..	....	.....sgKea	VRVLVNDaVq	PLEfCGg.vd
A. terreus 9a1	AYVEMMQCrA	....	..EK...EPL	VRVLVNDRVM	PLHGCPtDKL
A. terreus cbs	AYIEMMQCrA	....	..EK...QPL	VRVLVNDRVM	PLHGCAVDNL
A. niger var. *awamori*	lYVEMMQCQA	....	..EQ...EPL	VRVLVNDRVV	PLHGCPIDaL
A. niger T213	lYVEMMQCQA	....	..EQ...EPL	VRVLVNDRVV	PLHGCPIDaL
A. niger NRRL3135	lYVEMMQCQA	....	..EQ...EPL	VRVLVNDRVV	PLHGCPVDaL
A. fumigatus ATCC13073	AYfEtMQCKS	....	..EK...EPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus ATCC32722	AYfEtMQCKS	....	..EK...EPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus ATCC58128	AYfEtMQCKS	....	..EK...ESL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus ATCC26906	AYfEtMQCKS	....	..EK...EPL	VRaLINDRVV	PLHGCDVDKL
A. fumigatus ATCC32239	AYfEtMQCKS	....	..EK...EPL	VRaLINDRVV	PLHGCAVDKL
E. nidulans	AYfELMQCE.	....	..KK...EPL	VRVLVNDRVV	PLHGCAVDKF
T. thermophilus	AYIEMMQCDD	....	..sD...EPV	VRVLVNDRVV	PLHGCEVDsL
T. lanuginosa	AYVELLRCET	ETsSeEEeEG	..ED...EPF	VRVLVNDRVV	PLHGCrVDRW
M. thermophila	iYVEkMRCsG	GGgGgGGgEG	..rQekdEeM	VRVLVNDRVM	TLkGCGaDEr
Consensus Seq. 11	AYVEMMQCEA	GG-G-GG-EG	--EK---EPL	VRVLVNDRVV	PLHGCGVDKL

	451		482	
P. involutus (*phyA1*)	GlCtLAKFVE	SqTFARSDga	GDFEKCFAts	a~
P. involutus (*phyA2*)	GlCaLDKFVE	SqAYARSGga	GDFEKCLAtt	v~
T. pubescens	GvCtLDAFVE	SqAYARNDge	GDFEKCFAt~	--
A. pediades	SlCtLEAFVE	SqkYAReDgq	GDFEKCFD--	-~
P. lycii	GvCELsAFVE	SqTYAReNgq	GDFAKCgfvp	se
A. terreus 9a1	GRCKrDAFVA	GLSFAQAG..	GNWADCF---	--
A. terreus cbs	GRCKrDDFVE	GLSFARAG..	GNWAECF---	--
A. niger var. *awamori*	GRCtrDsFVr	GLSFARSG..	GDWAECsA--	--
A. niger T213	GRCtrDsFVr	GLSFARSG..	GDWAECFA--	--
A. niger NRRL3135	GRCtrDsFVr	GLSFARSG..	GDWAECFA--	--
A. fumigatus ATCC13073	GRCKLNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus ATCC32722	GRCKLNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus ATCC58128	GRCKLNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus ATCC26906	GRCKLNDFVK	GLSWARSG..	GNWGECFS--	--
A. fumigatus ATCC32239	GRCKLKDFVK	GLSWARSG..	GNSEQSFS--	--
E. nidulans	GRCtLDDWVE	GLNFARSG..	GNWktCFTl-	--
T. thermophilus	GRCKrDDFVr	GLSFARqG..	GNWEGCYAas	e~
T. lanuginosa	GRCRrDEWIK	GLTFARqG..	GHWDrCF---	--
M. thermophila	GmCtLErFIE	SMAFARGN..	GKWDlCFA--	--
Consensus Seq. 11	GRCKLDDFVE	GLSFARSG--	GNWAECFA--	--

Fig. 8D

```
        M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T       20
        ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC     60
   1    ---+---------+---------+---------+---------+---------+------
        TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

        A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C       40
        GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT     120
  61    ---+---------+---------+---------+---------+---------+------
        CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

        F  P  E  I  S  H  L  W  G  T  Y  S  P  Y  F  S  L  A  D  E       60
        TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA     180
 121    ---+---------+---------+---------+---------+---------+------
        AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

        S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q  V  L  S       80
        TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT     240
 187    ---+---------+---------+---------+---------+---------+------
        AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

        R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E       100
        AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA     300
 241    ---+---------+---------+---------+---------+---------+------
        TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAACTT

        A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N       120
        GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC     360
 301    ---+---------+---------+---------+---------+---------+------
        CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

        Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V  N  S  G       140
        TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGGTTAACTCTGGT     420
 361    ---+---------+---------+---------+---------+---------+------
        ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

        I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A       160
        ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT     480
 421    ---+---------+---------+---------+---------+---------+------
        TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

        S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A       180
        TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT     540
 481    ---+---------+---------+---------+---------+---------+------
        AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

        K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  N  V  I  I       200
        AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT     600
 541    ---+---------+---------+---------+---------+---------+------
        TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA

        P  E  G  S  G  Y  N  N  T  L  D  H  G  T  C  T  A  F  E  D       220
        CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC     660
 601    ---+---------+---------+---------+---------+---------+------
        GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG
```

Fig. 9A

```
     S  E  L  G  D  D  V  E  A  N  F  T  A  L  F  A  P  A  I  R     240
     TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTCGCTCCAGCTATTAGA
 661 ---+---------+---------+---------+---------+---------+------  720
     AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT

     A  R  L  E  A  D  L  P  G  V  T  L  T  D  E  D  V  V  Y  L     260
     GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
 721 ---+---------+---------+---------+---------+---------+------  780
     CGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGCAACAAATGAAC

     M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  E  L  S  P     280
     ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
 781 ---+---------+---------+---------+---------+---------+------  840
     TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

     F  C  A  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G     300
     TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
 841 ---+---------+---------+---------+---------+---------+------  900
     AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

     K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  A     320
     AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT
 901 ---+---------+---------+---------+---------+---------+------  960
     TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA

     N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H     340
     AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 ---+---------+---------+---------+---------+---------+------ 1020
     TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

     T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S     360
     ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021 ---+---------+---------+---------+---------+---------+------ 1080
     TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

     H  D  N  T  M  I  S  I  F  F  A  L  G  L  Y  N  G  T  K  P     380
     CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA
1081 ---+---------+---------+---------+---------+---------+------ 1140
     GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT

     L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T     400
     TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT
1141 ---+---------+---------+---------+---------+---------+------ 1200
     AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

     V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  Q  A  E  K  E  P     420
     GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA
1201 ---+---------+---------+---------+---------+---------+------ 1260
     CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT

     L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A  V  D  K     440
     TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG
1261 ---+---------+---------+---------+---------+---------+------ 1320
     AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACTGTTC

     L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G     460
```

Fig. 9B

```
     TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 ---+---------+---------+---------+---------+---------+------ 1380
     AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

     N  W  A  E  C  F  A  *   467
     AACTGGGCTGAATGTTTCGCTTAA
1381 ---+---------+---------+ 1410
     TTGACCCGACTTACAAAGCGAATT
```

Fig. 9C

```
                    M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T     20
                    ATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCACATCCGGTACC   60
               1    ---------+---------+---------+---------+---------+---------+
                    TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

                    A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G  Y  Q  C     40
                    GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT   -
              61    ---------+---------+---------+---------+---------+---------+  120
                    CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

A                   F  P  E  I  S  H  L  W  G  I  Y  S  P  F  F  S  L  A  D  E     60
                    TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA  180
             121    ---------+---------+---------+---------+---------+---------+
                    AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

                    S  A  I  S  P  D  V  P  K  G  C  R  V  T  F  V  Q  V  L  S     80
                    TCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTCAAGTTTTGTCT  240
             181    ---------+---------+---------+---------+---------+---------+
                    AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

                    R  H  G  A  R  Y  P  T  S  S  A  S  K  A  Y  S  A  L  I  E    100
                    AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA  300
             241    ---------+---------+---------+---------+---------+---------+
                    TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAACTT

                    A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K  T  Y  N    120
                    GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC  360
             301    ---------+---------+---------+---------+---------+---------+
                    CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

A                   Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  M  V  N  S  G    140
                    TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAACAACAAATGGTTAACTCTGGT  420
             361    ---------+---------+---------+---------+---------+---------+
                    ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA

                    I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F  I  R  A    160
                    ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT  480
             421    ---------+---------+---------+---------+---------+---------+
                    TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

                    S  G  S  D  R  V  I  A  S  A  E  K  F  I  E  G  F  Q  S  A    180
                    TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT  540
             481    ---------+---------+---------+---------+---------+---------+
                    AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA

                    K  L  A  D  P  G  A  N  P  H  Q  A  S  P  V  I  N  V  I  I    200
                    AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT  600
             541    ---------+---------+---------+---------+---------+---------+
                    TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA

                    P  E  G  A  G  Y  N  N  T  L  D  H  G  L  C  T  A  F  E  E    220
                    CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA  660
             601    ---------+---------+---------+---------+---------+---------+
                    GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT
```

Fig. 10A

```
      S  E  L  G  D  D  V  E  A  N  F  T  A  V  F  A  P  P  I  R    240
     TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTCCACCAATTAGA
 661 ---------+---------+---------+---------+---------+---------+  720
     AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

      A  R  L  E  A  H  L  P  G  V  N  L  T  D  E  D  V  V  N  L    260
     GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTTAACTTG
 721 ---------+---------+---------+---------+---------+---------+  780
     CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

      M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  T  Q  L  S  P    280
     ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA
 781 ---------+---------+---------+---------+---------+---------+  840
     TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

      F  C  D  L  F  T  H  D  E  W  I  Q  Y  D  Y  L  Q  S  L  G    300
     TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT
 841 ---------+---------+---------+---------+---------+---------+  900
     AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

      K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  V  G  F  V    320
     AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT
 901 ---------+---------+---------+---------+---------+---------+  960
     TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCAA

      N  E  L  I  A  R  L  T  H  S  P  V  Q  D  H  T  S  T  N  H    340
     AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
 961 ---------+---------+---------+---------+---------+---------+ 1020
     TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

      T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A  D  F  S    360
     ACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021 ---------+---------+---------+---------+---------+---------+ 1080
     TGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

      H  D  N  T  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  K  P    380
     CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCGGT

      L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  A  S  W  T    400
     TTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTGCTTCTTGGACT
1141 ---------+---------+---------+---------+---------+---------+ 1200
     AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

      V  P  F  A  A  R  A  Y  V  E  M  M  Q  C  E  A  E  K  E  P    420
     GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA
1201 ---------+---------+---------+---------+---------+---------+ 1260
     CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

      L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  G  V  D  K    440
     TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTTGACAAG
1261 ---------+---------+---------+---------+---------+---------+ 1320
     AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACTGTTC

      L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G    460
```

Fig. 10B

```
     TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 ---------+---------+---------+---------+---------+---------+ 1380
     AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

     N  W  E  E  C  F  A  *   467
     AACTGGGAAGAATGTTTCGCTTAA
1381 ---------+---------+---- 1404
     TTGACCCTTCTTACAAAGCGAATT
```

Fig. 10C

```
     M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T  S  G  T    20
     ATGGGGGTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCGGCAGCACATCGGGCACT
   1 ---------+---------+---------+---------+---------+---------+  60
     TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA

     A  L  G  P  R  G  N  H  S  K  S  C  D  T  V  D  L  G  Y  Q    40
     GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG
  61 ---------+---------+---------+---------+---------+---------+  120
     CGCGACCCGGGGGCACCTTTAGTGAGGTTCAGGACGCTATGCCATCTGGATCCCATGGTC

     C  S  P  A  T  S  H  L  W  G  T  Y  S  P  Y  F  S  L  E  D    60
     TGCTCCCCTGCGACTTCTCATCTATGGGGCACGTACTCGCCATaCTTTTCGCTCGAGGAC
 121 ---------+---------+---------+---------+---------+---------+  180
     ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG

     E  L  S  V  S  S  K  L  P  K  D  C  R  I  T  L  V  Q  V  L    80
     GAGCTGTCCGTGTCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA
 181 ---------+---------+---------+---------+---------+---------+  240
     CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT

     S  R  H  G  A  R  Y  P  T  S  S  K  S  K  K  Y  K  K  L  I    100
     TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt
 241 ---------+---------+---------+---------+---------+---------+  300
     AGCGCGGTACCTCGCGCCATGGGTTGGTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa

     T  A  I  Q  A  N  A  T  D  F  K  G  K  Y  A  F  L  K  T  Y    120
     ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTacGCCTTTTTGAAGACGTAC
 301 ---------+---------+---------+---------+---------+---------+  360
     TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCAtgCGGAAAAACTTCTGCATG

     N  Y  T  L  G  A  D  D  L  T  P  F  G  E  Q  Q  L  V  N  S    140
     AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACTCG
 361 ---------+---------+---------+---------+---------+---------+  420
     TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

     G  I  K  F  Y  Q  R  Y  K  A  L  A  R  S  V  V  P  F  I  R    160
     GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTCGC
 421 ---------+---------+---------+---------+---------+---------+  480
     CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

     A  S  G  S  D  R  V  I  A  S  G  E  K  F  I  E  G  F  Q  Q    180
     GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTCATCGAGGGGTTCCAGCAG
 481 ---------+---------+---------+---------+---------+---------+  540
     CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC

     A  K  L  A  D  P  G  A  T  N  R  A  A  P  A  I  S  V  I  I    200
     GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCCGCTCCGGCGATTAGTGTGATTATT
 541 ---------+---------+---------+---------+---------+---------+  600
     CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

     P  E  S  E  T  F  N  N  T  L  D  H  G  V  C  T  K  F  E  A    220
     CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG
 601 ---------+---------+---------+---------+---------+---------+  660
     GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACTCCGC
```

Fig. 11A

```
     S  Q  L  G  D  E  V  A  A  N  F  T  A  L  F  A  P  D  I  R      240
     AGTCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA 720
 661 ---------+---------+---------+---------+---------+---------+
     TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

     A  R  L  E  K  H  L  P  G  V  T  L  T  D  E  D  V  V  S  L      260
     GCTCGCctCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGTCAGTCTA 780
 721 ---------+---------+---------+---------+---------+---------+
     CGAGCGgaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

     M  D  M  C  P  F  D  T  V  A  R  T  S  D  A  S  Q  L  S  P      280
     ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCACCG 840
 781 ---------+---------+---------+---------+---------+---------+
     TACCTGTACACAgGCAAACTATGCCATCGCGCGTGGTCGCTGCGTTCAGTCGACAGTGGC

     F  C  Q  L  F  T  H  N  E  W  K  K  Y  D  Y  L  Q  S  L  G      300
     TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC 900
 841 ---------+---------+---------+---------+---------+---------+
     AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

     K  Y  Y  G  Y  G  A  G  N  P  L  G  P  A  Q  G  I  G  F  T      320
     AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC 960
 901 ---------+---------+---------+---------+---------+---------+
     TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCTATCCCAAGTGG

     N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S  T  N  S      340
     AACGAGCTGATTGCCCGGTTGACgCGTTCGCCAGTGCAGGACCACACCAGCACTAACTCG 1020
 961 ---------+---------+---------+---------+---------+---------+
     TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCACGTCCTGGTGTGGTCGTGATTGAGC

     T  L  V  S  N  P  A  T  F  P  L  N  A  T  M  Y  V  D  F  S      360
     ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA 1080
1021 ---------+---------+---------+---------+---------+---------+
     TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

     H  D  N  S  M  V  S  I  F  F  A  L  G  L  Y  N  G  T  E  P      380
     CACGACAACAGCATGGTTTCCATCTTCTTTGCATTGGGCCTGTACAACGGCACTGAACCC 1140
1081 ---------+---------+---------+---------+---------+---------+
     GTGCTGTTGTCGTACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG

     L  S  R  T  S  V  E  S  A  K  E  L  D  G  Y  S  A  S  W  V      400
     TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG 1200
1141 ---------+---------+---------+---------+---------+---------+
     AACAGGGCCTGGAGCCACCTTTCGCGGTTCCTTAACCTACCCATAAGACGTAGGACCCAC

     V  P  F  G  A  R  A  Y  F  E  T  M  Q  C  K  S  E  K  E  P      420
     GTGCCTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT 1260
1201 ---------+---------+---------+---------+---------+---------+
     CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTCAGCCTTTTCCTCGGA

     L  V  R  A  L  I  N  D  R  V  V  P  L  H  G  C  D  V  D  K      440
     CTTGTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG 1320
1261 ---------+---------+---------+---------+---------+---------+
     GAACAAGCGCGAAACTAATTACTGGCCCAACACGGTGACGTACCGACGCTACACCTGTTC

     L  G  R  C  K  L  N  D  F  V  K  G  L  S  W  A  R  S  G  G      460
```

Fig. 11B

```
     CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACCCCGCTACGTTCGACTTACTGAAACAGTTCCCTAACTCAACCCGGTCTAGACCCCCG

     N  W  G  E  C  F  S  *    467
     AACTGGGGAGAGTGCTTTAGTTGA
1381 ---------+---------+---- 1404
     TTGACCCCTCTCACGAAATCAACT
```

Fig. 11C

```
CP-1
   Eco RI  M  G  V  F  V  V  L  L  S  I  A  T  L  F  G  S  T
    TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
  1 ---------+---------+---------+---------+---------+---------+ 60
    ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

     S  G  T  A  L  G  P  R  G  N  S  H  S  C  D  T  V  D  G  G
    CATCCGGTACCGCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
 61 ---------+---------+---------+---------+---------+---------+ 120
    GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC
       CP-2
            CP-3
     Y  Q  C  F  P  E  I  S  H  L  W  G  Q  Y  S  P  Y  F  S  L
    GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
121 ---------+---------+---------+---------+---------+---------+ 180
    CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA

     E  D  E  S  A  I  S  P  D  V  P  D  D  C  R  V  T  F  V  Q
    TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTC
181 ---------+---------+---------+---------+---------+---------+ 240
    ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG
                  CP-4.7
                       CP-5.7
     V  L  S  R  H  G  A  R  Y  P  T  D  S  K  G  K  K  Y  S  A
    AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTgacTCTAAGggtAAGaagTACTCTG
241 ---------+---------+---------+---------+---------+---------+ 300
    TTCAAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCccaTTCttcATGAGAC

     L  I  E  A  I  Q  K  N  A  T  A  F  K  G  K  Y  A  F  L  K
    CTTTGATTGAAGCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
301 ---------+---------+---------+---------+---------+---------+ 360
    GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT
                                  CP-6
                                       CP-7
     T  Y  N  Y  T  L  G  A  D  D  L  T  P  F  G  E  N  Q  M  V
    AGACTTACAACTACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGG
361 ---------+---------+---------+---------+---------+---------+ 420
    TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

     N  S  G  I  K  F  Y  R  R  Y  K  A  L  A  R  K  I  V  P  F
    TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
421 ---------+---------+---------+---------+---------+---------+ 480
    AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA
                                              CP-8.7
                                                  CP-9
     I  R  A  S  G  S  S  R  V  I  A  S  A  E  K  F  I  E  G  F
    TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT
481 ---------+---------+---------+---------+---------+---------+ 540
    AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

     Q  S  A  K  L  A  D  P  G  S  Q  P  H  Q  A  S  P  V  I  D
    TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
541 ---------+---------+---------+---------+---------+---------+ 600
    AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC
```

Fig. 12A

```
                                                        CP-10.7
                                                             CP-11.7
      V   I   I   S   E   A   S   S   Y   N   N   T   L   D   P   G   T   C   T   A
    ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
601 ---------+---------+---------+---------+---------+---------+ 660
    TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC
```

Fig. 12B

```
      F  E  D  S  E  L  A  D  T  V  E  A  N  F  T  A  L  F  A  P
     CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTCGCTC
 661 ---------+---------+---------+---------+---------+---------+ 720
     GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG
                                                          CP-12.7

      A  I  R  A  R  L  E  A  D  L  P  G  V  T  L  T  D  T  E  V
     CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
 721 ---------+---------+---------+---------+---------+---------+ 780
     GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGtgacttC

     CP-13.7
      T  Y  L  M  D  M  C  S  F  E  T  V  A  R  T  S  D  A  T  E
     TTactTACTTGATGGACATGTGTtctTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
 781 ---------+---------+---------+---------+---------+---------+ 840
     AAtgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

      L  S  P  F  C  A  L  F  T  H  D  E  W  R  H  Y  D  Y  L  Q
     AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcacTACGACTACTTGC
 841 ---------+---------+---------+---------+---------+---------+ 900
     TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG
          CP-14.7
             CP-15.7
      S  L  K  K  Y  Y  G  H  G  A  G  N  P  L  G  P  T  Q  G  V
     AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
 901 ---------+---------+---------+---------+---------+---------+ 960
     TTAGAAACttcTTCATGATGCCAgtgCCACGACCATTGGGTAACCCAGGTtgaGTTCCAC

      G  F  A  N  E  L  I  A  R  L  T  R  S  P  V  Q  D  H  T  S
     TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
 961 ---------+---------+---------+---------+---------+---------+ 1020
     AACCAAAGCGATTGCTTAACTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
                     CP-16
                         CP-17.7
      T  N  H  T  L  D  S  N  P  A  T  F  P  L  N  A  T  L  Y  A
     CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG
1021 ---------+---------+---------+---------+---------+---------+ 1080
     GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

      D  F  S  H  D  N  G  I  I  S  I  F  F  A  L  G  L  Y  N  G
     CTGACTTCTCTCACGACAACggtattATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081 ---------+---------+---------+---------+---------+---------+ 1140
     GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
                               CP-18.7
                                    CP-19.7
      T  A  P  L  S  T  T  S  V  E  S  I  E  E  T  D  G  Y  S  S
     GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAAACTGACGGTTACTCTt
1141 ---------+---------+---------+---------+---------+---------+ 1200
     CATGACGAGGTAACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGAa

      A  W  T  V  P  F  A  S  R  A  Y  V  E  M  M  Q  C  Q  A  E
     ctgctTGGACTGTTCCATTCgcttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
1201 ---------+---------+---------+---------+---------+---------+ 1260
     gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTCGAC
                                              CP-20
                                                   CP-21
```

Fig. 12C

```
   K  E  P  L  V  R  V  L  V  N  D  R  V  V  P  L  H  G  C  A
    AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 ---------+---------+---------+---------+---------+---------+ 1320
    TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC
```

Fig. 12D

```
      V  D  K  L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R
     CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA
1321 ---------+---------+---------+---------+---------+---------+ 1380
     GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT
                                                      CP-22

      S  G  G  N  W  A  E  C  F  A  *  Eco RI
     GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 ---------+---------+---------+---------+------ 1426
     CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT
```

Fig. 12E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00154

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23K 1/165, A01H 5/00, C12N 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	EP 0619369 A1 (AVEVE N.V.), 12 October 1994 (12.10.94), See page 5, lines 11-15; page 7, lines 1-3; and claims 20-21 --	1-14
X	EP 0682876 A1 (SOUFFLET ALLMENTAIRE), 22 November 1995 (22.11.95), See page 3, lines 21-25 and claim 9 --	1-8
X	WO 9114782 A1 (GIST-BROCADES N.V.), 3 October 1991 (03.10.91), See page 2, lines 25-27 --	9-14

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 July 1999	13 -07- 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00154

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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01/06/99

International application No.
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